Chronic graft-versus-host disease (cGVHD), a multiorgan disorder, is the leading cause of late nonrelapse mortality (NRM) after hematopoietic stem cell transplantation (HSCT). Despite many years of experience with this disease we are still faced with numerous challenges including (among others); lack of reliable preclinical models, poor knowledge of human pathophysiology, validated diagnostic and severity criteria, and unpredictable clinical response to first line treatment. We will review recent advances on the 3 last mentioned unresolved areas.

**KEY WORDS:** Chronic GvHD, Pathophysiology, Diagnostic criteria, Treatment

**IMMUNE PATHOLOGY OF CHRONIC GRAFT-VERSUS-HOST DISEASE (J. RITZ)**

In contrast to acute graft-versus-host disease (aGVHD), chronic GVHD (cGVHD) often presents with clinical manifestations that resemble those of autoimmune diseases such as scleroderma, Sjögren’s syndrome, and systemic lupus erythematous (SLE). Nevertheless, relatively little is known about the immune mechanisms that lead to the development of cGVHD and how these might be similar or different than conventional autoimmune diseases. Despite having features of autoimmunity, cGVHD only occurs in the setting of allogeneic hematopoietic stem cell transplantation (HSCT). Moreover, both aGVHD and cGVHD can be prevented by depletion of T cells from the donor graft. Thus, donor T cells responding to allogeneic antigens in the recipient must play a central role in the development of cGVHD, but the precise immunologic targets of cGVHD are not well established and the immunologic mechanisms that lead to the distinctive but highly variable clinical features of this disease remain largely unknown.

**B and T Cell Responses in cGVHD**

Although donor T cells play a central role in the development of both aGVHD and cGVHD, recent studies have provided evidence that B cells also contribute to the clinical manifestations of this disease. In male patients who receive stem cell grafts from female donors, proteins encoded by a small number of genes on the Y chromosome have been shown to be targets of both T and B cell responses. In males, these proteins are self-antigens, and T cells specific for these antigens are deleted during thymic differentiation. Similarly, B cells specific for HY proteins are also deleted in males. In contrast, normal females are not exposed to these proteins and do not develop tolerance. When transplanted into male recipients, naive female lymphocytes encounter these Y-encoded proteins for the first time and respond to these proteins as though they were “foreign” antigens [1]. Approximately one-third of known minor histocompatibility antigens (miHA) are derived from this small set of genes on the Y chromosome, indicating that peptide epitopes from these proteins are readily processed and presented by both HLA class I and class II molecules. These peptide epitopes are readily recognized by the naïve female T cell repertoire, resulting in the clonal expansion of T cells specific for the targets of cGVHD.

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sex matched, miHA encoded by autosomal genes
are presumed to be the primary antigenic targets of
aGVHD and cGVHD, but relatively few of these epi-
topes have actually been described and further studies
are needed to identify and characterize the targets of
T cell immunity in patients with cGVHD.

Male patients who receive stem cell grafts from
female donors have provided a unique model in which
to characterize B cell responses to alloantigens after
HSCT. Whereas T cell responses are directed against
peptide epitopes presented by HLA class I and class II
molecules, antibody responses are directed against
soluble antigens. To characterize B cell responses
against miHA, we therefore tested plasma from
patients with cGVHD for IgG antibodies specifically
reactive with recombinant HY proteins by Western
blot and by ELISA [3,4]. HY antibodies were detected
in approximately 50% of male patients with female
donors compared to <10% of male patients with
male donors. HY antibodies typically develop 6 to 12
months after HSCT and are not associated with
aGVHD or other clinical variables. However, the
presence of these antibodies is significantly associated
with the development of cGVHD [4]. These findings
suggest that B cell responses to HY antigens also con-
tribute to the pathogenesis of cGVHD, but the
Y-encoded antigens that elicit this antibody response
are intracellular proteins and not expressed on the
cell membrane. It is therefore unlikely that antibodies
specific for these antigens are directly toxic to recipient
cells and the mechanisms whereby these antibodies
contribute to tissue damage remains to be elucidated.

B Cell Activating Factor (BAFF) and cGVHD

BAFF supports the differentiation and survival of
normal B cells, and elevated levels of BAFF in patients
with autoimmune diseases have been shown to pro-
mote the persistence of autoreactive B cells. Consider-
ing this important role of BAFF in B cell homeostasis,
our laboratory examined the role of BAFF in the
reconstitution of B cell immunity after HSCT. Soluble
BAFF was measured in plasma by ELISA, and initial
studies found that patients with active cGVHD had
significantly elevated levels of BAFF compared with
patients with resolved cGVHD or patients who never
developed cGVHD after transplant [5]. Prospective
monitoring of BAFF levels showed that they were
typically elevated in the first 3 months after HSCT.
In patients who reconstitute normal numbers of naïve
B cells, BAFF levels return toward normal. However,
BAFF levels remain elevated in the absence of recovery
of naïve B cells and persistently high levels of BAFF
promote the survival of activated CD27+ B cells.
The persistence of CD27+ activated B cells and high
levels of BAFF are both associated with the subsequent
development of cGVHD [6]. The observation that
donor B cells play a role in cGVHD has led to several
studies evaluating the safety and efficacy of B cell-di-
rected therapy with rituximab (anti-CD20) in patients
with steroid refractory cGVHD [7]. Several reports
have established the efficacy of this treatment, but
few complete responses (CR) are achieved with rituxi-
mab therapy and other B cell-directed approaches may
be more effective [8]. In this context, anti-BAFF agents
represent an alternative approach because reduction in
the levels of BAFF might provide a more specific and
less toxic way of reducing the chronic stimulation
and activation of reconstituting donor B cells and
suppressing secretion of alloreactive and potentially
autoreactive antibodies.

CD4+ Regulatory T Cells in cGVHD

Similar to autoimmune diseases, both T and B cell
responses appear to play a role in the pathogenesis of
cGVHD, suggesting that this reflects a general loss
of tolerance including abnormalities in the function of
CD4+ regulatory T cells (Tregs). Studies in mice
have clearly shown that Tregs are capable of suppress-
ing GVHD, and that deficiency of Tregs can lead to
increased severity of GVHD. Our previous analysis
of patients with cGVHD showed that these patients
had a relative deficiency of Tregs compared to patients
without GVHD [9]. To understand the mechanisms
responsible for this deficiency of Tregs we compared
the reconstitution of Tregs with conventional CD4+
T cells (Tcons) in the first year after transplant. During
this period, thymic generation of Tregs was signi-
ficantly impaired compared with Tcons, but Tregs
exhibited higher levels of endogenous proliferation.
Importantly, overall Treg reconstitution remained
low because this high level of proliferation was coun-
terbalanced by a greater susceptibility to apoptosis.
This homeostatic imbalance led to depletion of the
Treg population in peripheral blood and was associ-
ated with a high incidence and severity of cGVHD.

In summary, recent studies of immune reconstitu-
tion after allogeneic HSCT have provided several
novel insights into the mechanisms that lead to the
development of cGVHD. As clinical methods to modu-
late T, B, and Treg function become available, these
approaches can be applied in a rationale way to both
deplete alloreactive T and B cell populations and to
establish a normal level of peripheral tolerance [10].
These new approaches may provide more effective
treatment or strategies to prevent the development of
this important complication of allogeneic HSCT.

CURRENT ISSUES IN DISEASE DEFINITION
AND SEVERITY (G. SOCIE)

Disease Definition

It has been known for many years that, although
the disease usually manifests itself more than 100 days
after transplant, earlier disease onset could occur. More importantly, clinical syndromes with features of typical aGVHD are increasingly recognized beyond 100 days after HSCT especially in recent years with the development of reduced-intensity conditioning (RIC) regimens [11]. In addition, patients with aGVHD may progress to develop cGVHD with symptoms of both aGVHD and cGVHD. For many years, we have used a grading system, developed by the Seattle group [12], of limited versus extensive GVHD. This study was designed to identify patients needing systemic immune suppression, but does not capture the severity of individual organ involvement. Although other grading schemes have been proposed (reviewed in [13]) to predict survival following cGVHD, all lack consistent scoring and assessment of each organ involved to determine the overall severity of the disease. Recognizing these limitations, a group of experts lead by Dr. Pavletic at the NIH met in 2004 for a consensus conference on cGVHD. Because all participants agreed that it was urgently necessary to get rid of the formal definition of cGVHD (any GVHD beyond day 100), the diagnosis and staging working group of the NIH Consensus Development Project on cGVHD [14] proposed standard criteria for diagnosis (Table 1), organ scoring, and global assessment of cGVHD severity (see reference for the details on how to score organ severity and to assign a severity grade).

Usefulness of the NIH Criteria?

As of today, 5 groups worldwide have attempted to study patients who developed GVHD more than 100 days after allogeneic transplantation and to reclassify cGVHD using the NIH criteria. Two early studies, 1 by the Nashville group involving 110 patients [15], and another 1 from the Minneapolis group involving 54 patients [16], provided initial estimates. Classifications in both studies were late aGVHD (36% and 15%), overlap syndrome (26% and 28%), and classic cGVHD (37% and 57%) (estimates from [13]). Then Cho et al [17] studied 211 patients. Classifications were: late aGVHD (21%), overlap syndrome (30%), and classic cGVHD 49%.

Table 1. NIH Criteria for aGVHD and cGVHD

<table>
<thead>
<tr>
<th>Category</th>
<th>Time of symptoms</th>
<th>Presence of aGVHD Features</th>
<th>Presence of cGVHD Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic acute</td>
<td>≤100 D</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Persistent</td>
<td>&gt;100 D</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recurrent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late onset acute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cGVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic chronic</td>
<td>no time limits</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Overlap syndrome</td>
<td>no time limits</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

cGVHD indicates chronic graft-versus-host disease; aGVHD, acute graft-versus-host disease.

Classic cGVHD and overlap syndrome patients (n = 167) were graded using both the revised Seattle criteria and NIH global scoring. This large study addressed the critical point of what’s left with chronic GVHD using the stringent NIH disease criteria. Despite not being based on same patient number, and including different patient, disease, and transplant characteristics, one can thus reasonably assume that around 20% of patients formally classified as cGVHD using the Seattle day 100 landmark could, in fact, be considered as having features of an acute inflammatory disease. Most recently, the Seattle group addressed this issue in studying 740 patients who underwent HSCT after myeloablative (MA) conditioning and diagnosed with historically defined cGVHD. The presence or absence of NIH criteria for cGVHD showed no statistically significant association with survival, risks of nonrelapse mortality (NRM), or recurrent malignancy, or the duration of systemic treatment. Antecedent late aGVHD was associated with an increased risk of NRM and prolonged treatment among patients with NIH cGVHD. Authors concluded that these results support the consensus recommendation that, with appropriate stratification, clinical trials can include patients with late aGVHD as well as those with NIH cGVHD [18]. In an unpublished study, we at the Hospital St Louis retrospectively reviewed 116 consecutive patients undergoing an SCT for hematologic malignancy after RIC. After a median follow-up of 18 months, a total of 67 patients (58%) developed cGVHD according to the Seattle day 100 landmark criteria. When using NIH consensus criteria, 55 (47%) developed cGVHD (including 43 [53%] with classic cGVHD and 8 [10%] with overlap syndrome). Patients reclassified included; 3 patients with late-onset aGVHD, 19 with recurrent, and 8 with persistent aGVHD. The cumulative incidence of cGVHD at 36 months was 64% (95% confidence interval [CI]; 53%-73%) when using Seattle criteria compared to 56% (95% CI; 45%-67%) with NIH cGVHD criteria. Have those studies a purely semantic interest? I do not so believe. This means that all estimates currently published in the literature underestimate aGVHD incidence and overestimate that of cGVHD. This is not of major importance if you are aware of this caveat; however, it is of importance if you want to use these incidences to calculate the power of a clinical trial or if you want to search for a statistical link between either acute or cGVHD with relapse (graft-versus-leukemia [GVL] effect), for example.

Current Issues in Using the NIH Criteria

Ongoing unresolved issues include: (1) difference (if any) in GVHD-specific survival between in patient with late aGVHD compared to those with classical cGVHD or with the overlap syndrome? (2) Which...
classification better predict survival and discontinuation of immunosuppressive therapy? (3) Which classification will be a better discriminator of disease severity?

INITIAL TREATMENT OF CHRONIC GVHD (P.J. MARTIN)

In general, systemic immunosuppressive treatment is not needed for patients with mild manifestations of cGVHD involving a single organ, unless adverse risk factors are present. Risk factors associated with an increased risk of NRM include platelet count <100,000 at onset, treatment with prednisone at onset, antecedent aGVHD, and hyperbilirubinemia at onset. Systemic treatment is needed for patients with more severe manifestations, especially when multiple sites are involved. The treatments studied during the past 2 decades, have not demonstrably accelerated the resolution of cGVHD [19]. Survival at 5 years among patients with “standard-risk” cGVHD is approximately 70%, and survival among those with “high-risk” features of thrombocytopenia or antecedent aGVHD is approximately 50%.

Need for Glucocorticoid-Sparing Regimens in Treatment of cGVHD

Systemic glucocorticoids have long served as the mainstay of treatment for cGVHD. Initial doses of 1.0 mg/kg per day have been recommended, followed by tapering over a period of several months as allowed by improvement in disease manifestations. Long-term treatment with high-dose prednisone is associated with a high risk of complications and considerable morbidity. Some complications can be ameliorated by every other day administration of prednisone as opposed to daily administration. Effective glucocorticoid-sparing treatments would be of enormous benefit for patients with cGVHD, provided that the reduction in glucocorticoid-related side effects offsets any adverse effects of the treatment used to reduce the reliance on glucocorticoids. Benefits of effective initial treatment with glucocorticoid-sparing agents would be expected to include earlier discontinuation of immunosuppressive therapy, a reduced incidence of complications related to treatment with prednisone, a lower probability of secondary therapy, and a lower probability of death from causes other than recurrent malignancy.

Paucity of Randomized Trials for Treatment of cGVHD

Only 5 randomized trials [20–24] for systemic treatment of cGVHD have ever been reported (Table 2), and the principal clinical trials registration site in the United States currently lists only 7 phase II studies that are actively recruiting patients for systemic treatment of cGVHD. The historic paucity of phase III clinical trials for treatment of cGVHD reflects the difficulty of designing and conducting such studies. Virtually all previous treatment studies of cGVHD have been conducted with the use of agents that have been previously approved for other indications. Academic investigators have found it very difficult to attract the interest of industry partners, because cGVHD is an orphan indication with no established template for an approval path at the FDA. Identification of validated shorter term endpoints is critically important to overcome these obstacles and to attract the interest of industry partners toward investing in clinical trials related to cGVHD. Successful identification of validated short-term endpoints could create future opportunities to test novel agents that have not already been approved for other indications.

Lack of Validated Endpoints for Clinical Trial

Clinical trials in patients with cGVHD have been hampered by the lack of validated scales based on objective measurements that can be used to assess response and outcome after treatment, although some progress toward this goal has been made [25–27]. Many clinical trials have used “clinical improvement,” partial response (PR), or CR as endpoints, often at unspecified times after enrollment. As a more rigorous endpoint, some trials have used time to discontinuation of all immunosuppressive treatment as an indicator of cure, but ascertainment of this endpoint requires lengthy follow-up. Survival and mortality from causes other than recurrent malignancy have been used as unequivocal objective endpoints in clinical trials to measure the efficacy of treatment for cGVHD. Nonetheless, the interpretation of single-arm trials remains difficult because of potential selection bias. The key question is whether any shorter term endpoint could be used as a valid surrogate for longer term cure of the disease. Such endpoints would make it much easier to evaluate the merits of new approaches for treatment of cGVHD by shortening the timeline needed to assess outcomes and to identify promising new approaches.

Challenges for the Future

In a recently completed multicenter double-blinded trial [24], we found that the addition of mycophenolate

Table 2. Randomized Clinical Trials in cGVHD

<table>
<thead>
<tr>
<th>Author</th>
<th>Arms Compared</th>
<th>Double Blind</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sullivan [20]</td>
<td>Prednisone ± Azathioprine</td>
<td>Yes</td>
<td>Decreased survival</td>
</tr>
<tr>
<td>Koc [21]</td>
<td>Prednisone ± cyclosporine</td>
<td>No</td>
<td>Limited benefit</td>
</tr>
<tr>
<td>Koc [22]</td>
<td>Cyclosporine/Prednisone ± thalidomide</td>
<td>Yes</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Arora [23]</td>
<td>Cyclosporine/Prednisone ± thalidomide</td>
<td>No</td>
<td>No benefit</td>
</tr>
<tr>
<td>Martin [24]</td>
<td>Prednisone ± mycophenolate mofetil</td>
<td>Yes</td>
<td>No benefit</td>
</tr>
</tbody>
</table>

cGVHD indicates chronic graft-versus-host disease.
mofetil (MMF) to the initial immunosuppressive regimen used for treatment of cGVHD did not accelerate the resolution of cGVHD and withdrawal of systemic immunosuppressive treatment. We considered several explanations that might have accounted for the inability of MMF to produce favorable results, including an overstringent primary endpoint, suboptimal dosing of MMF, and inappropriate pacing of steroid withdrawal. Although data were limited, careful examination of results did not support any of these potential explanations. Hence, we concluded that MMF did not provide any overall benefit as a component in the initial treatment of cGVHD. By some measures, trends suggested that MMF may cause harm when added to conventional systemic immunosuppressive treatment for cGVHD.

This experience raises some major points for consideration by investigators interested in treatment of cGVHD. First, clinical trials should use randomized designs whenever possible. Numerous case-series reports and uncontrolled phase II studies suggested encouraging results with the use of MMF for treatment of steroid-refractory cGVHD, but our results with the use of MMF for initial treatment of cGVHD do not support the use of MMF for this indication. Second, despite concerted efforts to facilitate participation by physicians and patients at 15 centers, it took 4 years to enroll 157 patients in our study. Progress would be enhanced if physicians could more readily acknowledge the limitations of previous studies, thereby maintaining appropriate clinical equipoise with respect to unproved treatments. Third, clinical trials should involve collaborations between investigators and industry so that trials can use double-blinded designs to produce robust results. Blinding in cGVHD trials is especially important when objective measures remain difficult to document and when clinical endpoints unavoidably involve subjective judgment. Finally, improved understanding of the pathophysiology of cGVHD will be needed to develop more effective approaches for treatment. A challenge for the future will be the discovery of approaches that can target the cause of cGVHD while permitting reconstitution of immune defenses against pathogens and preserving immunologic effects of donor cells against malignant cells in the recipient.

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REFERENCES


