

B Cells and Transplantation: An Educational Resource

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) offers a unique opportunity to monitor the development of immune reconstitution using the patient as his or her own control. Furthermore, the donor serves as an HLA-identical normal control and source of adoptive immune transfer. Donor hematopoietic stem cells (HSCs) differentiate and proliferate to repopulate all blood lineages providing normal cell numbers of red blood cells, platelets, and neutrophils. Successful immune reconstitution and protection from infection requires antimicrobial B cell and antibody development. Studies of B cell reconstitution after HCT have primarily examined immunoglobulin concentration, B cell quantification, and antimicrobial antibody development in relation to donor/recipient serologic status or vaccination.

Following HCT, humoral immunity has 3 distinct contributions. First, recipient antibody persists with an average half-life of 30-60 days, and some recipient plasma cells persist for years following allogeneic HCT [1] providing protective antimicrobial humoral immunity [2]. Some recipient antidonor alloimmune responses are detrimental contributing to primary graft rejection [3,4] and prolonged red cell aplasia when donors and recipients are ABO major mismatched [5,6]. Second, donor grafts contain naïve and memory B cells that have already undergone positive and negative selection in the HLA-identical donor and contribute adoptive antimicrobial and alloreactive B cells. Third, B cells reconstituting from donor HSCs recognizing disparate recipient antigens as “self” will be clonally deleted, preventing alloreactive responses, but remain capable of responding to infectious challenges and vaccinations. This educational session will consider B cell responses following allo-

genic HCT as they contribute to (1) vaccine-induced antimicrobial immunity, (2) autoimmune responses, and (3) allogeneic antibody responses. We will discuss a B cell role in chronic graft-versus-host disease (cGVHD) pathogenesis, review anti-B cell cGVHD therapy using rituximab, and, finally, consider the pathogenic role of agonistic antibodies targeting platelet-derived growth factor receptor (PDGFR).

Normal B Cell Ontogeny

B cell development is schematically depicted in Figure 1. Progenitor B cells receive signals from essential bone marrow stromal cells via cell-cell contacts and secreted signals. Stem cell factor (SCF) on stromal cell membranes binds ckit (CD117) on the lymphocyte membrane, and secreted cytokines, especially interleukin (IL)-7, promote B cell development [7-9]. B cells bind antigen with varying affinity through B cell receptors that gain diversity through intrachromosomal variable (V) and constant (C) region recombination [10]. B cell positive selection requires tonic signaling through membrane pre-B receptor and membrane IgM expression for the B cell to survive. Mouse knock-out experiments expressing null alleles of the heavy chain transmembrane exon, Iga or Igb genes, or their ITAMs prevents B cell development [11,12]. Likewise, productive somatic recombination leads to allelic exclusion for both heavy and light chains in each individual B cell. B cells recognizing self antigens are negatively selected before emerging from the bone marrow.

Accumulating data suggests the BCR affinity “threshold” is influenced by cytokine tumor necrosis factor (TNF) family member B cell-activating factor (BAFF; also termed BLyS). Three receptors have been identified that bind to BAFF: transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI); B cell maturation Ag (BCMA); and BAFF-R. Baff-R(-/-) mice mount significant, but reduced, Ag-specific Ab responses [13]. BAFF and its receptors, play a crucial role in peripheral B cell selection and survival, by dictating the set point for the number of mature primary B cells and adjusting thresholds for specificity-based selection during transitional differentiation [14,15]. Transgenic models demonstrate that antigen-induced anergy and exclusion from follicular niches of autoreactive B cells depends on the

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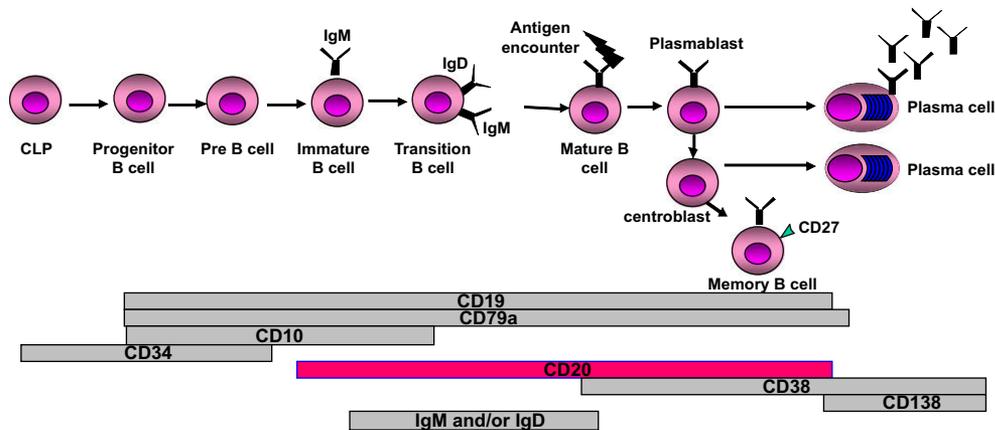


Figure 1. B cell maturation profile.

presence or absence of a diverse B cell pool [16]. In addition, B cell reconstitution and homeostasis after myeloablation requires the B survival factor BAFF [17]. Limiting amounts of BAFF are required for ongoing B cell turnover and avoidance of B cell autoreactivity [18]. This is because in the setting of a limited B cell pool, excess BAFF promotes the survival of autoreactive B cells [19]. These BAFF homeostatic demands suggest a paradigm that unites peripheral negative and positive selection with the maintenance of mature B cell numbers [20,21] that probably have an impact on post-HCT reconstitution. Plasma BAFF levels are markedly elevated following myeloablative conditioning and decrease as lymphocyte numbers recover. Elevated BAFF has been associated with cGVHD [22] and autoimmune diseases [23-25].

Antibody Reconstitution after HCT

Early studies showed IgG and IgM return to normal concentrations 3-4 months after allogeneic HCT [26,27], whereas B cells are quantitatively deficient during the first month and persists in some patients for more than a year after allo-HCT [28-30]. Antibody assessment is complicated by blood product support transferring significant immunoglobulin and antibody half-life extending 30-60 days. Nonetheless, vaccination with neoantigens phage ϕ X174 and keyhole limpet hemocyanin (KLH) demonstrated effective IgG responses 6 months after HCT, but antibody response lagged in patients with cGVHD or those who received antithymocyte globulin (ATG) [31]. A reduced immunoglobulin repertoire persists for at least 2 years after HCT with oligoclonal dominance [32,33]. The diversity of heavy IgG VDJ gene rearrangement reflects a broad adult μ -chain usage arguing against a recapitulation of fetal ontogeny [34]. Post-HCT IgH repertoire is characterized by decreased somatic hypermutation [35], delayed class-switching [36], and oligoclonal dominance [37,38].

Analysis of herpes simplex virus (HSV) and cytomegalovirus (CMV) clarified that antiviral Ab responses

were of recipient origin for the first year post-HCT [2,39,40]. Persistent high-titer IgG responses persisted when recipients were seropositive and donors seronegative, and likewise recipients remained seronegative even when the donor was seropositive unless viral infection erupted [41]. Deliberate vaccination of donors pretransplant with a protein recall antigen, such as tetanus toxoid [42], or neoantigen KLH [43], could demonstrate adoptive B cell transfer but antibody development still required recipient revaccination after transplant.

Donor and Recipient Antibody Contribution Can Be Distinguished by Allotype

Allotype-specific monoclonal antibodies (mAbs) specifically bind single amino acid polymorphisms located in the immunoglobulin constant fragment of specific IgG isotypes [44]. If either the donor or recipient is homozygous null for an allotype then the HCT pair is informative allowing antibody origin to be determined. For example, if the recipient is null for IgG1 detection using allotype reagent, then the development of donor derived IgG1 is allotype detected. Such allotype reagents provided the first evidence of donor B cell reconstitution after HCT [45], and that some recipient plasma cells persist for years after myeloablative HCT. In a pediatric study, 8 of 13 informative pairs showed some persistent recipient IgG more than a year and some 8 years after myeloablative allogeneic HCT [1]. More recently, IgG allotype studies showed recipient antimicrobial IgG predominates following reduced-intensity conditioning (RIC) HCT, and DNA chimerism confirmed that the majority of bone marrow plasma cells remained recipient derived 1 year after RIC allo-HCT [46]. In support of this recipient humoral immunity predominance, a study of 87 patients randomized to receive either marrow or peripheral blood stem cells (PBSCs) showed vaccine-specific antibody responses in the first year after allo-HCT reflected primarily the recipient's pretransplant titer [47]. Thus, humoral immunity is predominately

recipient derived for the first year after RIC allogeneic HCT, and this persistent recipient derived antimicrobial IgG may benefit RIC allo-HCT patient and contribute to their decreased treatment related mortality (TRM).

Immunogenicity of Vaccines following HCT

Vaccine preventable infections, such as pneumococcus [48], influenza [49], and varicella [50] remain a significant cause of morbidity, rehospitalization, and mortality after successful HCT. Despite the fact that influenza infections remain a significant cause of serious upper and lower respiratory tract infections post-HCT, many patients do not receive their yearly influenza immunization. Most studies report a 30% to 40% incidence of reactivation of varicella zoster virus (VZV) following HCT. In a retrospective study of 100 consecutive allogeneic HCT recipients, 41% developed a VZV infection at a median of 227 (range: 45–346) days posttransplantation [50]. Forty percent of patients required admission to the hospital, with a mean stay of 7.2 days. Postherpetic neuralgia and peripheral neuropathy developed in 68% of patients [18]. As such, revaccinating HCT patients prevents significant morbidity and mortality, and, in addition, well-designed vaccine studies functionally assess B cell immune reconstitution.

Protective Immunity Dissipates after Myeloablative HCT

Multiple studies have demonstrated that in the absence of revaccination, both autologous and allogeneic transplant recipients lose seroprotection to pathogens they were immunized against during childhood (reviewed in [51,52]). Although there is some variability in the time to protective titer loss among different transplant groups, loss of pneumococcal, H flu, and tetanus titers usually occur by 2 years post-HCT [53]. Residual titers against measles, mumps, and rubella persist longer after HCT [54]. These vaccine studies showing the loss of humoral immunity studied myeloablative HCT patients, therefore, RIC studies are needed.

Vaccine Lessons

Repetitive vaccination improves efficacy

Following an HLA matched related bone marrow transplant (BMT), Ljungman et al. [55] showed that 42%, 36%, and 21% of patients immunized with 1 inactivated polio virus vaccine (IPV) developed a 4-fold rise in titer against serotypes 1, 2, and 3, respectively. Following 3 doses, 50% of patients responded to all 3 serotypes. In contrast, Parkkali et al. [53] reported a 100% rate following 3 IPV whether administered at 6, 8, and 14 months (n = 23) or at 18, 20, and 26

months (n = 22). At Memorial Sloan Kettering Cancer Center (MSKCC), 96% of 219 allogeneic HCT recipients responded to a series of 3 IPV when administered following acquisition of minimal milestones of immune competence (CD4 >200/ μ L, PHA within 10% of normal, IgG >500 mg/dL) [56]. There was no difference in response in related or unrelated HCT recipients, or those who received a T cell-depleted or T cell-replete HCT. The range of responses in these 3 studies may reflect in part variable levels of immune competence at the time of revaccination.

Vaccine efficacy varies by glycosylation and conjugation

Although most transplant patients respond well to vaccines containing bacterial toxoids, response to pure polysaccharide vaccines has been poor [57–60]. Barra et al. [57] demonstrated that only 4 of 20 (25%) adult allogeneic transplant recipients immunized with a single nonconjugated H flu vaccine developed a specific IgG response compared to 11 of 20 patients recipients 1 H flu conjugate vaccine (Hib) ($P < .05$). Most studies have also shown that <25% of HCT recipients are capable of responding to the polyvalent polysaccharide vaccine, PPV23 [57–59], as well as no benefit of donor vaccination with PPV23 prior to stem cell donation [60]. Limited response to polysaccharide antigens post-HCT may be because of the predominance of immature B cells expressing CD1c, CD5, CD38, CD23⁺, B cells similar to the circulating B cells detected in cord blood and young children [29]. Limited responses may also reflect deficits in CD27⁺ memory B cells, a subset that may take years to recover even in children following HCT [61]. Nonetheless, heavy-chain IgG sequence analysis of Hib-specific B cells collected after Hib revaccination 9 months after myeloablative allogeneic BMT showed unique patterns of hypermutation, suggesting 90 of 121 (74%) were derived from only 16 precursors, and 12 of these clones were identified in the donor [62]. Thus, this single-patient study suggests memory B cells specific to Hib were transferred from the donor, persisted 9 months, and contributed the majority of Hib-specific repertoire.

In contrast to the poor response to polysaccharide vaccines, response to protein-conjugated polysaccharide vaccines is significantly better, and can be enhanced by donor vaccination prior to the stem cell harvest [63,64]. Meisel et al. [65] showed a 74% response to the 7 serotypes contained in PCV7 in 43 patients <17 years of age immunized with 3 PCV7 starting at 6 months following a related or unrelated HCT. A retrospective study of PVC7 and Hib responses in 127 patients immunized at MSKCC also demonstrated a decline in PCV7 response with advancing age [66]. Forty-five of 51 patients <18 years

of age responded to PCV compared to 34 of 76 adults ($P < .001$). Although PCV7 response was adversely affected by older age ($P < .001$), individuals ≥ 50 years old responded significantly better if vaccinated following acquisition of specific minimal milestones of immune competence, CD4 $>200/\mu\text{L}$, IgG $>500 \text{ mg/dL}$, or PHA within 60% lower limit of normal (11 of 19 versus 0 of 8, $P < .006$). A similar trend was observed in patients with limited cGVHD. Each of these studies demonstrates that PCV7 is immunogenic in HCT patients, including older adults, but suggest that vaccination timing might depend on immune competence for best vaccine response.

Vaccine efficacy is predicted by recipient immune reconstitution status

Revaccination against hepatitis B is mandatory for reentry to school and certain workplaces. Machado et al. [67] reported a 100% seroconversion rate in 50 HCT recipients immunized at least 1 year after transplant. Despite this excellent initial response, 60% of patients failed to sustain titers for more than 1 year after vaccination. MSKCC evaluated the response of 267 allogeneic transplant recipients immunized with rHBV following acquisition of minimal milestones of immune competence [56]. Sixty-four percent of patients seroconverted, including 73% of 99 children and 59% of 168 adults ($P = .02$). In multivariate analyses, response was adversely affected by age >18 years ($P < .01$) and history of prior cGVHD ($P < .0001$). Eighty-two percent of 99 evaluated patients remained seropositive 5 years following their last vaccine. This greater proportion of patients with sustained hepatitis B titers in the MSKCC (82%), compared to Machado and colleague’s study (40%), suggests qualitative and/or quantitative differences in the circulating memory T and/or B cells present at the time of vaccination. Surrogate markers of immune competency may be necessary in determining the need and timing of booster immunizations to maintain durable protective titers following vaccination.

Vaccine guidelines

The above data demonstrates that unlike vaccination in healthy individuals, vaccination post-HCT does not ensure seroprotection, emphasizing the need to document pre- and postvaccine titers to determine response. Although vaccination of patients with limited or no cGVHD will likely respond well to immunization starting 6 months post-HCT, how best to protect patients requiring steroids \pm other immunosuppressive agents, is currently not known. Table 1 presents the consensus vaccination guidelines of the Center for Disease Control (CDC) [57] and the European Blood and Marrow Transplant [58], and Center for International Blood and Marrow Transplant Re-

Table 1. Consensus Vaccination Guidelines

Vaccine	CDC	EBMT
Td, IPV, Hib	2 doses Started 12m	3 doses Started 6-12m
PPV23	12 m, 24 m	12m
HepB	Optional	Recommended
MMR	24 months	24 months
Varivax	NO	NO

search (CIBMTR) guidelines will soon be issued. Prospective trials documenting the efficacy of these guidelines to protect the growing numbers of transplant survivors are needed.

B cell Reconstitution following Allogeneic HCT

Quantitative B cell deficiency in peripheral blood has long been noted in the first month after HCT and can persist for years being worsened by cGVHD and/or the treatment of cGVHD [28-30,70]. Figure 2 shows that peripheral blood CD19⁺ B cell reconstitution in pediatric and adult recipients has similar kinetics but varies by graft source with T cell depleted bone marrow products preceding unmanipulated, T cell replete bone marrow grafts (T. Small data).

Peripheral blood B cell development is currently characterized by 4 surface markers: CD19, IgD, CD38, and CD27 immunophenotyping. The results of normal donor CD19⁺ gated B cells are presented as 2-dimensional comparison of IgD versus CD38 in Figure 3. The IgD versus CD38 comparison provides the Bm1-Bm5 classification that identifies: (a) virgin naïve cells (IgD⁺CD38⁻), (c) pregerminal center cells or transitional (IgD⁺CD38⁺⁺), (b) postgerminal center memory cells (IgD⁻CD38^{+/-}), and (d) germinal center plasmablasts (IgD⁻CD38⁺⁺), reviewed [71]. The IgD versus CD27 classification provides meaning because CD27 is the universal marker of “memory” and thus distinguishes between CD27⁻IgD⁺ naïve B cells, CD27⁺IgD⁺ “class able to switch” memory

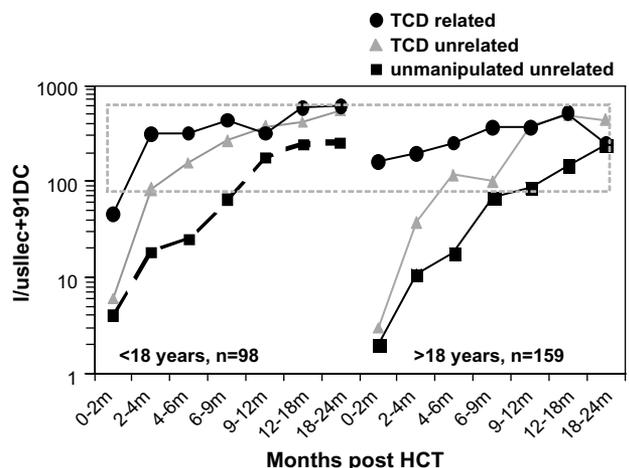


Figure 2. Median number of CD19⁺ B cells/ μL peripheral blood after allogeneic HCT. Kindly provided by Trudy Small.

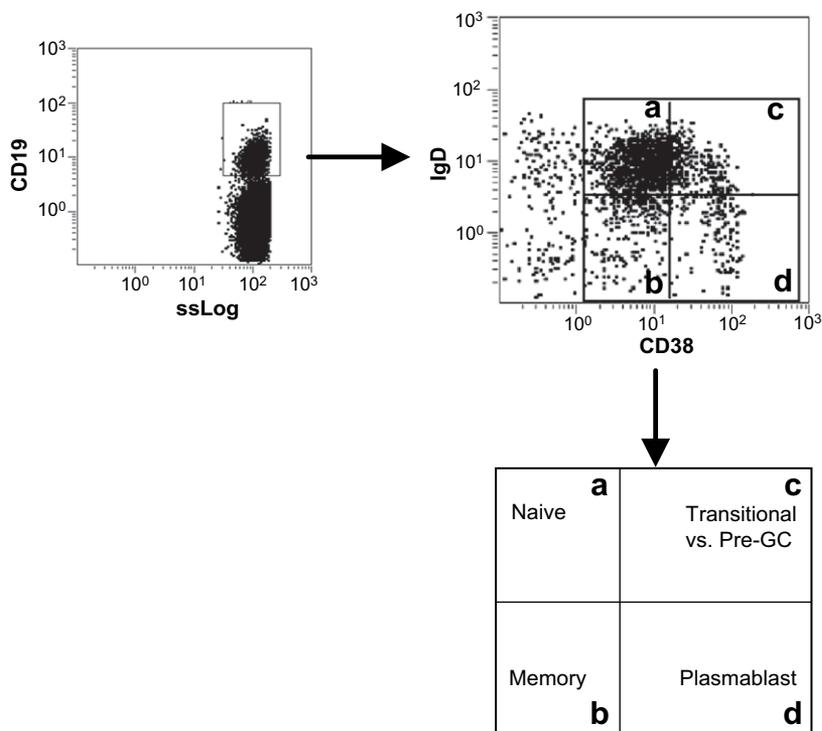


Figure 3. Peripheral B cell immunophenotyping based on the expression of IgD and CD38 shows normal donor peripheral blood B cells are mostly naïve.

cell, and CD27⁺IgD⁻ “isotype switched” memory cells. Increased CD27 expression on human B cells is known to correlate with commitment to the plasma cell lineage [72]. B cells develop in bone marrow and the immunophenotype of the earliest bone marrow emigrant to the peripheral blood is the IgD⁺CD38⁺CD27⁻ “transitional” cell, which is the precursor to the “naïve” mature IgD⁺CD38⁻ cell. Progression then moves to pregerminal center founder (Pre-GC) CD27⁺ and postgerminal center memory cell. Both the pre-GC and post-GC can become IgD⁻ isotype switched plasmablasts. The pre-GC are IgD⁺CD38^{Hi}CD27⁺ cells are typically found in human tonsils [73], and have been shown to circulate with increased frequency in systemic lupus erythematosus (SLE) patients [74,75]. Sarantopoulos et al. [76] analyzed peripheral CD27⁺ B cell subsets in 57 post-HCT patients and healthy controls by performing 4 color-flow cytometry analyses. CD27⁺ was increased on all IgD versus CD38 populations in patients with cGVHD compared to patients without cGVHD. Pre-GC B cells were more prevalent in patients with active cGVHD than those without cGVHD ($P = .06$) [76].

A longitudinal study of 139 pediatric HCT recipients undergoing myeloablative (119 allogeneic) HCT showed recovering B cells at 3, 6, and 12 months after HCT are predominately naïve (IgD⁺CD27⁺), and consequently, a significantly lower percentage of both “class able to switch” (IgD⁺CD27⁺) and “class

switched” (IgD⁻CD27⁺) B cells when compared to age-matched controls [63]. Even at 18 to 24 months after HCT there was a significant number of patients with CD27⁺ memory cells less than the fifth percentile of normal controls. As predicted by the lack of memory B cells, an in vitro T cell-independent polyclonal stimulation revealed reduced IgG production, which has also been reported in adults [70]. Neither B cell subset distribution nor stimulated Ig production correlated with source of stem cells, type of conditioning, immune suppression, or the development of acute GVHD (aGVHD) or cGVHD. Avanzini et al. [63] propose that germinal center B cell reactions are disturbed post-HCT possibly because of lymph node histoarchitectural damage. Additional B cell immunophenotyping studies may identify abnormal developmental immunophenotypes especially in the setting of elevated BAFF possibly accounting for autoantibody production and functional immune deficiencies after HCT.

B Cells and cGVHD

Mouse studies suggest a role for B cells in the development of scleroderma and cGVHD. B lymphocytes have been implicated in cutaneous sclerosis in both murine and human studies. The tight skin (Tsk^{+/+}) mouse has a tandem duplication within the fibrillin-1 (FBN1) gene and heterozygous mice exhibit increased collagen and other matrix protein deposits in their skin [77]. If bone marrow and splenocytes are

transplanted from Tsk/+ donors into normal mice, a scleroderma phenotype and autoantibodies develop [78,79]. B cell depletion using an antimouse CD20 mAb before or 3 days after birth suppressed skin fibrosis by 43% and autoantibody production [80]. Studies in minor histocompatibility antigen mismatched mouse models of cGVHD support involvement of B cells in its pathogenesis [81-83].

In humans, Miklos et al. [84] demonstrated allogeneic antibodies against at least 1 Y chromosome encoded protein (H-Y antigen) developed in 52% of female → male recipient HCT tested by ELISA against a 5 H-Y antigen panel: DDX3y, UTY, ZFY, RPS4Y, and EIF1AY. In the presence of antibodies to at least 1 H-Y protein, the cumulative incidence of cGVHD reached 89% at 5 years after transplantation compared to only 31% in the absence of H-Y antibodies ($P < .0001$) [85]. The concordant development of allogeneic H-Y antibodies and chronic GVHD provided early evidence for an important B cell role in chronic GVHD pathogenesis.

A possible role for elevated soluble BAFF in cGVHD is plausible because high BAFF levels are found in patients with autoimmune diseases [25,86], including scleroderma [87], and murine models of B cell autoimmunity. In mice, both normal and autoreactive B cell development depends on the relative balance of B cell receptor (BCR) and BAFF signaling [88-92]. In the setting of a limited B cell pool, excess BAFF promotes the survival of autoreactive B cells [19]. Sarantopoulos et al. [22] studied BAFF levels in 104 allogeneic HCT patients and showed BAFF levels were significantly higher in patients with active cGVHD, compared to those without disease ($P = .0002$). Serial testing of 24 HCT patients showed BAFF levels were high in the first 3 months following HCT, but subsequently decreased in 13 patients who never developed cGVHD. In contrast, BAFF remained elevated in 11 patients who developed cGVHD. BAFF >10 ng/mL 6 months after allogeneic HCT was strongly associated with subsequent cGVHD development [22]. One complication of this analysis is that BAFF levels are suppressed in patients receiving more than 30 mg of prednisone daily, and prednisone is almost always used to treat aGVHD and cGVHD.

Rationale for Rituximab Treatment of cGVHD

Rituximab, a humanized IgG1 antibody against CD20, depletes B cells and is effective in treating patients with steroid-refractory cGVHD. Cutler et al. [91] reported a phase I clinical trial testing Rituximab in 21 patients with steroid-refractory cGVHD. Rituximab (375 mg/m²/week × 4 weeks) was given with an option for a second course for non- or partial responders. Rituximab was well tolerated, and objective responses were noted in 13 of 20 patients (70%, primarily cutaneous and rheumatologic). CD19⁺ B cells

were undetectable in all peripheral blood samples for 9 to 12 months; median serum IgG levels fell 37%, but protective IgG antibody responses against Epstein Barr virus (EBV) EBNA1 and tetanus toxoid remained unchanged in 17 out of 18 patients. Antibody titers against H-Y antigens decreased after rituximab therapy and remained low for a year after treatment. All 4 patients with demonstrable H-Y antibodies had clinical responses to rituximab therapy [93].

Zaja et al. [94] reported a 65% overall response rate in 38 patients with steroid-refractory cGVHD treated with rituximab. Skin was the most likely to respond (63%) followed by mouth (48%), eyes (43%), lung (38%), and liver (25%). Infections were the major complication. Mohty et al. [95] treated 15 severe or steroid refractory cGVHD patients with rituximab weekly for 4 weeks, and responders received 1 to 2 courses of maintenance rituximab. With a median follow-up of 118 days from first rituximab infusion, no major toxicities were ascribed to rituximab. Overall, 10 patients (66%) responded, and 3 achieved complete responses. Four patients did not respond and died of refractory cGVHD.

As shown schematically in Figure 1, CD20 (developmental expression of CD20 shown in red) is first expressed on B cells after they have undergone heavy- and light-chain recombination and express IgM BCR on their cell surface, a stage called “immature B cells.” CD20 continues to be expressed until the cell becomes an immunoglobulin secreting plasma cell. T cell-dependent human plasma cells in secondary lymphoid tissue are CD20⁺ [96]. Thus, rituximab treatment postallogeneic HCT may potentially eliminate CD20⁺ alloreactive cells while leaving early pre- and pro-B cells to develop into nonalloreactive mature B cells. Rituximab therapy is well tolerated with relatively few infectious complications [93] because long-lived host plasma cells continue to secrete protective antimicrobial antibodies [97].

Autoantibodies Are Associated with Systemic Sclerosis

Systemic sclerosis involves progressive fibrosis with obliteration of small artery lumens as well as humoral immunologic dysregulation associated with hypergammaglobulinemia and antinuclear antigen antibodies (ANA) [98]. These autoantibodies include antitopoisomerase I (Scl70), anticentromere, anti-RNA polymerase III, anti-U3-fibrillarin, and others [99]. However, despite their association with scleroderma, these ANA have not been shown to be pathogenic. Other autoantibodies targeting extracellular matrix proteins include: antifibrillin-1 (anti-FBN1) [100] and antimatrix metalloproteinases (MMP), such as MMP1 (interstitial collagenase) [101] and MMP3 (stromelysin) [102]. FBN1 is a 350-kDa glycoprotein that is the major constituent of microfibrils in the

extracellular matrix and they sequester transforming growth factor (TGF)- β . Anti-FBN-1 antibodies are present in the majority of scleroderma patients, and some authors suggest anti-FBN1 antibodies may release TGF- β , which can then activate fibroblasts promoting fibrosis [103,104].

Because the clinical manifestations of cGVHD share many features with scleroderma [105], cGVHD patients have been repeatedly examined for autoantibody development with inconsistent results. Some studies suggest autoantibodies develop in association with cGVHD [106,107], whereas others show autoantibodies develop as frequently as 25% after allogeneic transplantation but occur equally in patients with and without cGVHD [108].

Antibodies against PDGFR Associate with Systemic Sclerosis and cGVHD

Fibroblasts have been extensively investigated as the target of autoantibodies in patients with scleroderma [109]. PDGF receptors are upregulated in the skin and bronchoalveolar lavage fluid in scleroderma [110]. In 2006, Svegliati et al. [111] reported 46 patients with systemic sclerosis had stimulatory antibodies against PDGFR. These antibodies were absent in 20 healthy controls and another 55 patients with a variety of other rheumatologic conditions. In another study, agonistic PDGFR antibodies were detected in 22 allogeneic HCT patients with extensive cGVHD. Anti-PDGFR antibodies were not detected in 17 HCT patients without cGVHD and 20 normal controls [112]. These PDGFR- α antibodies induced tyrosine phosphorylation, accumulation of reactive oxygen species (ROS), and type 1 collagen gene expression through the Ha-Ras-ERK1/2-ROS signaling pathway, all processes implicated in inflammation and fibrosis [113]. PDGFR antibodies are relatively long lived compared to the 15-minute half-life of PDGF itself.

Thus, anti-PDGFR agonistic antibodies may be the pathogenic cause of sclerosis in some cGVHD patients, and drug inhibition of PDGFR signaling is a promising treatment for sclerosis. This might be achieved through either direct tyrosine kinase inhibition of PDGFR or anti-B cell therapy that eliminates the agonistic anti-PDGF antibody. Drs. Lorinda Chung and Bill Robinson have initiated a phase I clinical trial of imatinib treatment of systemic sclerosis with promising preliminary results (Chung et al, manuscript submitted). Immunohistochemical analysis of serial skin biopsies obtained before and 1 month after imatinib 200 mg/day treatment show decreased anti-phospho-PDGFR antibody staining in the setting of clinical improvement. Extending this treatment strategy to cGVHD, the Italian bone marrow transplant cooperative group, GITMO, reported a safety and tolerability study of imatinib (100-200 mg daily) for cGVHD as an abstract at the European Bone Marrow

Transplant Meeting in April 2008 [114]. They reported an overall response of 86% with a follow-up of 8 months. Aspects of cGVHD that responded to imatinib included sclerodermatous disease, chronic bronchiolitis, and osteomyalgia. Toxicity was evaluated at 3 and 6 months. Four of 15 subjects experienced minor extrahematologic toxicity and 1 of 15 experienced grade 3-4 toxicity. Taken together, the frequent association of cGVHD and anti-PDGFR antibody and reported imatinib safety and tolerability in cGVHD patients supports further imatinib efficacy trials to determine cGVHD response rate and confirm its mechanism of action.

Future Directions

Protective antimicrobial immunity and allogeneic immune responses ultimately results from the reconstitution of donor lymphocytes with diverse T and B cell repertoires following allogeneic HCT. Nonetheless, humoral immunity immediately post-HCT is predominately recipient derived. Over the past 10 years, RIC regimens have decreased treatment-related mortality (TRM) and extended HCT to older patients. The success of these RIC allogeneic HCT relies primarily on developing beneficial allogeneic immune responses, graft-versus-leukemia/lymphoma (GVL), and effective protective immune reconstitution. Although RIC regimens have succeeded in decreasing TRM and aGVHD incidence, cGVHD remains problematic. One key difference between high-dose conditioning and RIC is the dynamic balance between the decreasing host-versus-graft (HVG) resistance to engraftment and the developing graft-versus-host (GVH) immune responses. Although myeloablative HCT causes rapid conversion to full donor T and B cell chimerism, RIC patients' progress through a transient mixed chimerism extending weeks to months, and the pace of transition differs by conditioning regimen [115]. Thus, we believe an improved understanding of B cells and serologic immune responses following RIC HCT in relation to antimicrobial immunity, GVL, and GVHD are critical. Future vaccine studies will functionally assess B cell immune reconstitution following RIC allogeneic HCT thereby decreasing patients' infectious complications and revealing B cell transplant biology. B cell immunophenotyping, heavy-chain IgG repertoire analysis, and B cell functional assays combined with multiplexed serologic antigen binding assays promises to identify allogeneic antibody and B cell contributions to both GVL and GVHD.

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