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B-cell-directed therapy for chronic graft-*versus*-host disease

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Although acute morbidity and mortality associated with allogeneic hematopoietic stem cell transplantation have steadily decreased over the past 20 years, chronic graft-*versus*-host disease (GVHD) remains a common complication and few new treatment approaches have been identified during this period. Unfortunately, therefore, chronic GVHD remains a frequent long-term toxicity that often affects patients who might otherwise be cured of their primary disease. Our current knowledge of the immune pathophysiology of chronic GVHD is limited in part because few animal models have been developed that mimic the varied clinical manifestations of this disease in humans.[1] The effective prevention of chronic GVHD by the depletion of T cells from the stem cell graft demonstrates that donor T cells play a critical role in this disease. When patients and donors are HLA-matched, minor histocompatability antigens expressed in normal tissues of the recipient have been shown to elicit both $CD4^+$ and $CD8^+$ T-cell responses which result in either direct cell killing or cytokine-induced tissue injury. Thus, current therapies for chronic GVHD are targeted primarily and non-specifically against donor T-cell activity.

Although donor T cells play a central role in the development of chronic GVHD, there is emerging evidence that donor B cells also contribute to the clinical manifestations of this disease.[2] In a mouse model of major histocompatability complex-mismatched transplantation, donor B cells were found to be necessary for the development of chronic GVHD.[3] In humans, at least some of these B cells produce known autoantibodies.[4] Our laboratory has previously shown that Y chromosome-encoded minor histocompatability antigens elicit specific antibody responses following sex-mismatched hematopoietic stem cell transplantation, and the presence of these allo-antibodies correlates with the development of chronic GVHD.[5,6] Patients with minor histocompatability antigen-specific antibodies have also been found to have alloreactive $CD4^+$ T cells directed against different epitopes derived from the same protein[7] but the pathogenicity of these antibodies remains unproven. Several mechanisms whereby donor B cells can interact

with T cells and contribute to the immune pathology of chronic GVHD are listed in Table 1. In addition to mechanisms mediated by specific antibodies, these include direct antigen-presentation, the production of cytokines that modulate the intensity and type of immune response, and the development of immunoregulatory B cells that suppress T-cell responses.[2]

Rituximab, an anti-CD20 monoclonal antibody, has shown promise in treating steroid-refractory chronic GVHD, further supporting a role for B cells in the development of this disease. Initial retrospective studies identified small numbers of patients who responded to rituximab,[8,9] and their findings were subsequently confirmed by larger, dedicated studies.[10,11] A meta-analysis of published rituximab studies in patients with steroid-resistant chronic GVHD revealed an overall response rate of 66%, with responses observed for disease involving the skin, oral mucosa, liver, and lung.[12] Although specific decreases in antibodies directed against Y chromosome-encoded minor histocompatability antigens have been identified in some patients, the kinetics of clinical response after rituximab treatment suggest that responses often occur before antibody titers are affected.[10] Rituximab, which results in profound B-cell depletion, may, therefore, be helpful against both antibody-dependent and antibody-independent B-cell mechanisms of chronic GVHD.

In this issue of Haematologica, Kim *et al.*[13] report the results of a prospective, multicenter phase II trial of weekly rituximab followed by monthly rituximab maintenance therapy for the treatment of steroid-refractory chronic GVHD. With 37 patients, this trial is the largest prospective trial of rituximab for chronic GVHD published to date and is the only study to include both pediatric and adult patients. The authors report an overall response rate of 86%, with a complete response rate of 25%. Fifty-seven percent of the patients were able to reduce or discontinue steroid use by 1 year. Although this is the first trial to incorporate maintenance treatment with rituximab, response rates and the ability to reduce steroids after 1 year of therapy were similar to those reported previously. The median time to maximal response was shorter than that in previous studies (29 *versus* 46-138 days) and although decreases in immunglobulins (IgA, IgM, and IgG) were observed, these were not measured until day 57. Thus it remains unclear whether rituximab is effective by counteracting antibody-dependent or antibody-independent B-cell activity. The fall in immunoglobulin levels may, however, be important as seven patients (19%) developed pneumonia or sepsis during the follow-up period; this is similar to what has been observed in other studies with rates of sepsis and pneumonia being 3-33% and 8-33%, respectively.[12] The infectious complications of rituximab in this setting are, therefore, significant, and require immunoglobulin monitoring as well as consideration of antibiotic prophylaxis. Defining the necessary and effective dose of rituximab for the therapy of chronic GVHD may be one way to minimize the risk of infections. One group utilized low-dose rituximab (50 mg/m^2 weekly for 3 weeks) for treatment of chronic GVHD and reported response rates that were similar to those of other trials in which higher doses were used (375 mg/m^2 weekly for 4 weeks).[14] However, infectious complications were also observed with low-dose rituximab, suggesting that further studies are needed to define the optimal dose and schedule of rituximab for the treatment of steroid-refractory chronic GVHD.

Table 1. Possible mechanisms of B-cell involvement in chronic graft-vs-host disease.

B-cell action	Effector mechanism
Alloantibody production	Complement activation, antibody-dependent cellular cytotoxicity (ADCC), or cross-presentation of immune complexes
Antigen presentation	Priming of CD4$^+$ and CD8$^+$ T cells
Cytokine production	Shaping the type and strength of immune response by activation and recruitment of other immune cells
Immune regulation	Potential loss of peripheral T-cell tolerance through depletion or loss of regulatory B-cell subset

In their current study, Kim *et al.*[13] found higher levels of B-cell activating factor (BAFF) in patients with active chronic GVHD at baseline than in normal controls. Although not statistically significant, patients with lower pre-treatment BAFF levels tended to have better outcomes from therapy. Serum BAFF levels increased as B-cell numbers and immunoglobulin levels fell in response to rituximab therapy. This is consistent with the known function of BAFF as an important regulator of B-cell homeostasis and survival, and BAFF has previously been shown to play a critical role in B-cell reconstitution following myeloablative conditioning.[15] At normal basal levels, BAFF serves to promote survival of antigen-specific B cells; however, persistently high BAFF levels are also able to prevent apoptosis of auto-reactive B cells and promote the development of autoimmunity.[16] Earlier work from our laboratory showed that BAFF levels are higher in patients with chronic GVHD than in those without.[17,18] Patients with chronic GVHD also have low numbers of B cells, and high BAFF/B cell ratios are, therefore, characteristic of active chronic GVHD. Patients who do not develop chronic GVHD during the first year after hematopoietic stem cell transplantation have higher numbers of B cells at 6 and 9 months post-transplant than patients who do develop chronic GVHD. Further phenotypic analysis revealed that patients without chronic GVHD have higher proportions of naïve CD27$^-$ B cells whereas patients who develop chronic GVHD have higher proportions of activated CD27$^+$ B cells. These findings suggest that persistent elevation of BAFF in the setting of delayed B-cell reconstitution can support the survival of activated, allo-reactive B cells and, therefore, promote the development of chronic GVHD. BAFF is also produced by myeloid cells in the setting of inflammation, and this may be another factor driving ongoing BAFF production once B-cell numbers recover in patients with chronic GVHD.[19] Interestingly, high doses of corticosteroids have been shown to lower BAFF levels, and this may represent one of the mechanisms by which these agents lead to improvements in chronic GVHD.

As summarized in Table 1, there are several potential mechanisms through which donor B cells can contribute to the clinical manifestations of chronic GVHD. In most of these mechanisms, B cells do not act independently but modulate immune responses of other cells, primarily CD4$^+$ and CD8$^+$ T cells. For example, alloantibodies can form

immune complexes with recipient minor histocompatability antigens and incorporation of these immune complexes by dendritic cells can stimulate donor T-cell responses specific for these minor histocompatability antigens. Extensive depletion of all mature B cells with rituximab should interrupt all of the pathways in which B cells interact with T cells and suppress both antibody-dependent and antibody-independent B-cell mechanisms associated with chronic GVHD. It should, however, be noted that similar interactions between B and T cells have also been proposed for graft-*versus*-leukemia responses.[20] The profound B-cell depletion that occurs with rituximab may, therefore, also affect graft-*versus*-leukemia responses, and clinical studies of rituximab in chronic GVHD should also closely monitor relapse as a potential adverse consequence of this treatment approach.

What we have learnt about the role of B cells in chronic GVHD and B-cell immune reconstitution following hematopoietic stem cell transplantation may allow us to develop new targeted therapeutic approaches that limit global immune suppression and improve both disease-related and treatment-related outcomes. For example, neutralizing anti-BAFF antibodies have shown pre-clinical efficacy in animal models of autoimmune disease and belimumab, a monoclonal anti-BAFF antibody, was recently tested in phase III clinical trials in patients with systemic lupus erythematosus with encouraging results.[21] This antibody has not been tested in chronic GVHD but belimumab or other agents targeting BAFF may offer a more selective approach for suppressing some of the B-cell functions that contribute to sustained alloimmunity. Understanding the role of specific alloantibodies, pathogenic B-cell subsets or regulatory B cells may offer new opportunities to manipulate the B-cell compartment in ways that decrease alloimmunity while maintaining an otherwise effective immune system. Providing effective therapy for chronic GVHD without compromising normal immune functions and the graft-*versus*-leukemia effect remain important challenges and goals for new therapeutic approaches.

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