B-cell involvement in chronic graft-versus-host disease

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ABSTRACT

Chronic graft-versus-host disease is a serious complication in long-term survivors of allogeneic hematopoietic stem cell transplantation, with several organ systems affected. Chronic graft-versus-host disease is an important cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation. This article reviews the pathogenesis of chronic graft-versus-host disease. In particularly, the role of B cells in chronic graft-versus-host disease is evaluated, as is evident from several studies which have investigated the presence of antibodies as well as studies which have analyzed B cells as a target for immunotherapy. Thirty autoantibodies and 5 alloantibodies have been identified in chronic graft-versus-host disease patients in 24 studies, and 8 autoantibodies and 5 alloantibodies seemed to be strongly associated with chronic graft-versus-host disease. In addition, various studies have observed significant improvements in chronic graft-versus-host disease using the anti-CD20$^+$ antibody rituximab. However, it appears to be highly likely that both B cells as well as T cells are of major importance in chronic graft-versus-host disease. Further research is required to clarify the pathogenesis of chronic graft-versus-host disease.

Key words: B cells, chronic graft-versus-host disease, autoantibodies, rituximab.

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Introduction

Chronic graft-versus-host disease (cGVHD) is the most common problem affecting long-term survivors of allogeneic hematopoietic stem cell transplantation (HSCT), and is a significant cause of morbidity and mortality in patients receiving HSCT.[1] cGVHD is becoming increasingly more prevalent; as more patients survive transplantation, and more transplantation procedures are being performed with peripheral blood stem cells, as recipient age increases at transplantation, and with increased use of alternative donors.[2-4] cGVHD can affect several organ systems, as presented in Table 1. The most widely employed first-line therapy for cGVHD is a combination of cyclosporine A and prednisone. However, patients who fail to respond to steroid-based therapy have a poor outcome.[5] Therefore, various agents have been exploited as salvage therapy, but consensus has yet to be reached.

Interestingly, many autoimmune diseases such as primary biliary cirrhosis (PBC), Sjögren's syndrome and scleroderma have several features in common with cGVHD,[6,7] as regards to clinical manifestations as well as to the prevalence of autoantibodies. In PBC anti-mitochondrial antibodies (AMA) are the serological hallmark of the disease, being present in 90-95% of the patients.[8] In addition, antinuclear antibodies (ANA)[9-15] and elevated IgM levels are also found.[8] Regarding Sjögren's syndrome, autoantibodies include anti-Ro/SSA and anti-La/SSB,[16,17] ANA,[17-20] anti-α-fodrin antibodies,[21] antibodies directed against acethylcholine receptors[22] and anti-islet cell autoantigen 69 (ICA69) antibodies.[23] In addition, anti-parietal cell antibodies, AMA, anti-ribonucleoprotein antibodies, anti-smooth muscle (anti-Sm) antibodies without any significant clinical or analytical associations, and rheumatoid factor (RF) have also been reported.[17] The international consensus criteria for Sjögren's syndrome state that at least one of the autoantibodies anti-Ro/SSA, anti-La/SSB or IgM rheumatoid factor, is included as one of the criteria in order to confirm the diagnosis.[24] Concerning scleroderma, anti-endothelial cell antibodies,[25,26] anti-fibrillin-1 antibodies,[27] anti-matrix metalloproteinases 1 and 3[28,29] and anti-platelet-derived growth factor receptor (PDGFR) antibodies[30,31] have been described. Treatment of PBC is generally initiated with ursodeoxycholic acid (ursodiol), and colchicine

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Table 1. Overview of organ systems which can be affected by cGVHD, including clinical signs/laboratory findings and histopathological features. Adapted and modified from Higman *et al.*[1]

Organ system	*Clinical signs/laboratory findings*	*Histopathology*
Skin (common)	Hyper- and hypopigmentation, lichen planus, poikiloderma, cutaneous ulcers, scleroderma, icthyosis, bullous pemphigoid.	Lichenoid: hyperkeratosis, focal hypergranulosis, acanthosis, dyskeratotic keratinocytes, vacuolar degeneration, colloid bodies, perivascular and periadnexal lymphoplasmacellular infiltrate. Poikiloderma: epidermal atrophy, loss of rete ridges. Scleroderma: epidermal atrophy, dermal fibrosis, less inflammation than lichenoid lesions, destruction of adnexal structures.
Cutaneous structures	Onchodystrophy, alopecia, loss of sweat glands.	Destruction and fibrosis of cutaneous appendages.
Mouth (common)	Lichen planus, erythema, ulcers, xerostomia, dental caries, fibrosis, decreased salivary flow.	Mucosal atrophy, lymphoplasmacytic inflammation, increased mucopolysaccharides, fibrosis and destruction of minor salivary glands.
Eye (common)	Keratoconjunctivitis sicca, corneal ulcerations.	N.d.
Liver (common)	Icterus, elevated alkaline phosphatase, transaminases, bilirubin.	Small bile duct atypia and damage with subsequent necrosis and dropout, moderate lymphocytic infiltrate, cholestasis and ballooning.
Lung	Obstructive more than restrictive abnormalities on pulmonary function testing, bronchiolitis obliterans, pneumothoraces, bronchiectasis, pseudomonal colonization, pulmonary infiltrates.	Bronchiolitis obliterans with granulation tissue plugs and fibrosis obliterating small airways, interstitial pneumonitis.
Musculoskeletal	Polymyositis, arthritis, fasciitis.	Biopsy non-specific but may demonstrate muscle fiber dropout, usually transudative.
Esophagus	Esophageal web, desquamation, ulcerations, strictures, submucosal fibrosis, abnormal motility.	N.d.
Intestines	Fibrosis, malabsorption	N.d.
Serous	Pericardial, peritoneal and pleural effusions.	N.d.
Nervous	Entrapment of nerves, peripheral neuropathy, myasthenia gravis.	N.d.
Urological	Cystitis, phimosis.	N.d.
Renal (rare)	Glomerulonephritis.	Biopsy may demonstrate focal segmental proliferative glomerulonephritis with glomerulosclerosis and large cellular crescents in glomeruli.
Vagina	Erythema, lichen-planus like, sicca, strictures, stenosis, ulcers.	N.d.
Hematopoietic	Thrombocytopenia, neutropenia, eosinophilia, hemolytic anemia, pure red cell aplasia.	N.d.
Immuno	Lymphoid hypocellularity, hyper- or hypogammaglobulinemia.	N.d.

N.d: not described in the original article.

and methothrexate can be added.[8] Therapy for Sjögren's syndrome includes topical agents in order to moisterize and decrease inflammation. In addition, therapy for extraglandular manifestations involves systemic therapy like steroids or other anti-inflammatory agents, disease-modifying drugs and cytotoxic drugs. Treatment of sclerodermatic skin lesions include ultraviolet-A light therapy, glucocorticoids, calcipotriol and methotrexate. Several studies have found clinical features of Sjögren's syndrome to improve after treatment with rituximab, an anti-CD20 antibody, mostly including Sjögren syndrome-related lymphoma.[32-45] No studies to date have analyzed the influence of rituximab in patients with PBC or scleroderma.

The involvement of autoantibodies and alloantibodies as well as the effect of rituximab in cGVHD will be discussed in the following section. This article addresses the pathogenesis of cGVHD, which is not yet completely understood, with a special focus on the possible role of B cells.

Pathogenesis of cGVHD

B cells

Twenty-four research groups have investigated the involvement of antibodies in the pathogenesis of cGVHD, and found 35 different antibodies to be more prevalent in cGVHD (Table 2). Although not always analyzed, 8 autoantibodies and 5 alloantibodies seemed to be strongly associated with cGVHD in clinical terms, i.e. disease severity, namely the autoantibodies anti-cytoskeletal intermediate filament antibodies,[46] anti-cytoplasmic squamous epithelium antibodies,[47] anti-nucleolar B23 antibodies,[48,49] anti-nucleolar C23 antibodies,[48,49] anti-H1 histones antibodies,[48,49] anti-nuclear lamins A/C antibodies,[48,49] anti-thyroid microsome antibodies,[47] anti-PDGFR antibodies,[50] and the alloantibodies anti-DBY antibodies,[51-53] anti-UTY antibodies,[53] anti-ZFY antibodies,[53] anti-RPS4Y antibodies,[53] anti-EIF1AY antibodies.[53] Interestingly, most studies have tested different antibodies.

Table 2A. Overview of identified antibodies (autoantibodies) to date present in cGVHD patients.

Autoantibody	*Prevalence in cGVHD patients after allogeneic HSCT*	*Degree of association with cGVHD*
ANA	82% (n=28)[106]	–
	38% (n=26)[107]	+++
	62% (n=53)[108]	–
	25% (n=21)[109]	–
	22% (n=89)[110]	?
	23% (n=13)[111]	++
	43% (n=63)[112]	–
Anti-mitosin antibodies	100% (n= 1)[113]	++
Anti-Sm antibodies	82% (n=28)[106]	–
	35% (n=26)[107]	?
	49% (n=53)[108]	–
	17% (n=21)[109]	–
	26% (n=89)[110]	?
	15% (n=13)[111]	+
	6% (n=63)[112]	?
AMA	14% (n=28)[106]	–
	11% (n=53)[108]	–
	82% (n=11)[114]	++
	6% (n=63)[112]	?
Anti-epidermal antibodies	14% (n=28)[106]	–
	11% (n=53)[108]	–
Anti-cytoskeletal intermediate filaments antibodies	100% (n=16)[46]	+++
Anti-cytoskeleton antibodies	91% (n=11)[98]	++
IgM anti-cytoplasmic factor	37% (n=19)[115]	–
Anti-cytoplasmic squamous epithelium antibodies	42% (n=36)[47]	+++
Anti-double stranded	15% (n=26)[107]	?
DNA (anti-dsDNA)	31% (n=13)[111]	–
antibodies	3% (n=63)[112]	?
Anti-nucleolar antibodies	23% (n=26)[107]	?
	22% (n=63)[112]	–
Anti-nucleolar B23 antibodies	45% (n=22)[48,49]	+++
Anti-nucleolar C23 antibodies	27% (n=22)[48,49]	+++
	8% (n=37)[70]	+
Anti-H1 histones antibodies	18% (n=22)[48,49]	+++
Anti-nuclear lamins A/C antibodies	9% (n=22)[48,49]	+++
Anti-liver kidney microsome antibodies	6% (n=53)[108]	–
Anti-thyroid microsome antibodies	43% (n=40)[47]	+++
	5 % (n=63)[112]	?
Anti-interferon α (INF-α) antibodies	100% (n=1)[116]	?
Anti-CD13 antibodies	79% (n=19)[117,118]	?
Anti-topoisomerase I antibodies	11% (n=37)[70]	+
Anti-polymyositis/scleroderma (PM/Scl) antibodies	5% (n=37)[70]	+
Anti-La/SSB antibodies	3% (n=37)[70]	?
Anti-neutrophil cytoplasmatic	24% (n=21)[109]	–
antibodies (ANCA)	17% (n=47)[119]	?
	11% (n=63)[112]	?
p-ANCA	100% (n=1)[120]	++
Anti-reticulin antibodies	22% (n=21)[109]	–
RF	10% (n=21)[109]	–
	8% (n=13)[111]	–
Anti-cardiolipin antibodies	10% (n=89)[110]	?
(ACLA)	2% (n=63)[112]	?
Anti-heat shock protein (hsp) 70 IgM antibodies	48% (n=27)[121]	++
Anti-hsp90 IgM antibodies	48% (n=27)[121]	++
Anti-PDGFR antibodies	100% (n=22)[50]	+++

Table 2B. Overview of identified antibodies (alloantibodies) to date present in cGVHD patients.

Alloantibody	*Prevalence in cGVHD patients after allogeneic HSCT*	*Degree of association with cGVHD*
Anti-DBY antibodies	100% (n=1)[51]	? (F → M HSCT)
	50% (n=60)[52]	+++ (F → M HSCT)
	47% (n=75)[53]	+++ (F → M HSCT)
Anti-UTY antibodies	24% (n=75)[53]	+++ (F → M HSCT)
Anti-ZFY antibodies	16% (n=75)[53]	+++ (F → M HSCT)
Anti-RPS4Y antibodies	5% (n=75)[53]	+++ (F → M HSCT)
Anti-EIF1AY antibodies	8% (n=75)[53]	+++ (F → M HSCT)

Prevalence of antibodies in cGVHD patients after allogeneic HSCT, with n representing the number of patients studied. Degree of association with cGVHD: ? unknown association, - no association, + weak association, ++ positive association, +++ strong association, F → M HSCT: male allogeneic HSCT patients with female donors.

Not much is known regarding the pathogenicity of autoantibodies and alloantibodies in cGVHD patients. Despite over 20 years of investigation, no studies have convincingly demonstrated that these antibodies contribute to the clinical manifestations of tissue injury in cGVHD. The location of most target antigens of these antibodies is intracellular and the antibodies do not have a direct cytolytic activity. In addition, tissue injury via immune complexes or vasculitis is not probable, as these are not prominent features of cGVHD. However, recently it has been demonstrated that sera from 46 patients with scleroderma contained antibodies to the PDGFR.[30] This led to a signal transduction cascade involving activation of Ha-Ras, extracellular signal-regulated kinase (ERK)1/2, and reactive oxygen species (ROS). Eventually, this resulted in an increased type I collagen-gene expression and myofibroblast phenotype conversion in normal human primary fibroblasts. This fibroblast activation is a characteristic feature of scleroderma. Interestingly, Svegliati *et al.* have described the same phenomenon in 22 patients with extensive cGVHD, where higher levels of anti-PDGFR antibodies were detected in patients with generalized skin involvement and/or lung fibrosis.[50] These antibodies were also shown to activate the Ha-Ras, ERK1/2, ROS signal transduction cascade, leading to increased type I collagen-gene expression. Therefore, these autoantibodies might play a causal role in the pathogenesis of sclerodermatous cGVHD, ultimately leading to fibroblast activation. Nevertheless, the contribution of anti-PDGFR antibodies with respect to the clinical manifestations of tissue injury in cGVHD still remains to be established.

T cells

Traditionally, the main focus has been on T cells, which are considered to be major effectors and initiators in cGVHD. These T cells could attack tissue directly through cytolytic attack, secretion of cytokines inducing inflammation or fibrosis. Recently, T-regulatory cells (Tregs) have been investigated. Tregs can suppress proliferation and function of T cells, par-

ticularly of the Th1 type.[54] They are also known to constitutively express CD25+.[55] Using a murine model, it was demonstrated that the incidence and severity of cGVHD is higher in the absence of recipient CD4+ CD25+ T cells, and the subsequent repletion with recipient or host Tregs resulted in a protective effect.[56] In addition, monitoring of Foxp3 expression as a marker of Tregs, showed Treg-deficiency in cGVHD patients.[57] In contrast, another study of 17 patients showed high numbers of CD4+ CD25+ T cells in cGVHD patients.[58] Therefore, the function of Tregs in cGVHD is undetermined. In addition, in a murine model it was demonstrated that *de novo* generation of donor CD4+ T cells during acute GVHD is of importance for the progression to cGVHD.[59] Furthermore, various T-cell depleting antibodies, including antithymocyte globulin,[60] alemtuzumab (campath-1H, anti-CD52+ antibody)[61-66] and basiliximab (anti-CD25+ antibody),[67] have been shown to be effective in preventing cGVHD. However, these were all small, phase I and II, single center studies. Therefore, a prospective controlled randomized multicenter study is highly warranted to confirm these promising results.

Co-ordinated B- and T-cell response

Several studies have suggested a possible collaboration between B and T cells in the pathogenesis of cGVHD. This is in accordance with the fact that T-helper cells, via CD40 ligand expression, are necessary for immunoglobulin isotype switching.[68] Additionally, treatment with rituximab led to an incomplete response in a bullous pemphigoid-cGVHD patient.[69] But when adding daclizumab, an anti-CD25 antibody, a complete clinical response in combination with a complete decrease in bullous pemphigoid titer was observed. Daclizumab may have interrupted the helper function of CD4+ T cells, which facilitate the secretion of autoantibodies against bullous pemphigoid antigen 2 (BPAG2) by CD20- plasma cells. In addition, various studies demonstrated B-cell responses to certain antigens in cGVHD patients, indicating the collaboration of B and T cells to produce specific antibodies against host antigens.[48,52,53,70] Moreover, Zorn *et al*. demonstrated a coordinated B- and T-cell response in a male cGVHD patient after allogeneic HSCT with a female donor.[51] In this study, donor B cells were shown to mediate an alloimmune response and donor CD4+ T cells mediated an autoimmune response, via the development of anti-DBY antibodies. Furthermore, in cGVHD patients treated with rituximab, total lymphocytes decreased even more severely in number than B cells, suggesting that rituximab may somehow suppress T cells that interact with B cells.[71] This seems to be enigmatic, considering the fact that T cells are CD20-. In contrast, Canninga-van Dijk *et al*. found no changes in T cells and T-cell subsets after treatment with rituximab.[72] Moreover, using a murine model of cGVHD, it was recently demonstrated that cGVHD (sclerodermatous and glomerulonephritis) induction required both donor CD4+ CD25- T cells and B cells.[73] Interestingly, donor CD4+ CD25+ T cells (Tregs) prevented the induction of cGVHD. This indicates a strategy consisting of depletion of CD4+ CD25- T cells as well as B cells and infusion of donor Tregs, in order to prevent cGVHD. The necessity of infusion of donor Tregs can be supported by another study, in which T-cell depletion did not reduce the incidence of cGVHD or improve survival in cGVHD patients.[74]

Dendritic cells

The evidence concerning a role for dendritic cells in the pathogenesis of cGVHD is limited. Previously, studies had shown the association of early donor dendritic cell reconstitution with the non-appearance of severe GVHD, as the immune system should switch from host type to donor type following allogeneic HSCT in order for hematopoiesis to be regenerated.[75,76] One study demonstrated a role for host dendritic cells in the development of cGVHD, as from day 100 after allogeneic HSCT the persistence of host dendritic cells appeared to be correlated with the onset of severe acute GVHD and cGVHD.[77] Interestingly, another study reported that modified dendritic cells with an increased capacity for immune response regulation, known as regulatory dendritic cells, have a protective effect regarding incidence and severity of cutaneous cGVHD in a murine model, via generation of alloreactive CD4+ CD25+ Foxp3+ regulatory T cells (Tregs).[78]

Treatment of cGVHD with rituximab

Based on the fact that B cells might possibly produce pathogenic antibodies in cGVHD, it can be envisaged that B cells may provide a new target for immune intervention in cGVHD. In B-cell malignancies (lymphoma), rituximab has been developed as a treatment-strategy. Rituximab is an anti-CD20 chimeric mouse-human IgG antibody which enhances B-cell lysis via complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and apoptosis. Moreover, rituximab has also proven its efficacy in several autoimmune diseases, including Sjögren's syndrome[32-45] and systemic lupus erythematosus (SLE).[79-85] As cGVHD also appears to have autoimmune features, it can be argued that rituximab might be effective in the treatment of cGVHD. For this purpose, a number of studies have analyzed the potential of rituximab in patients with cGVHD and have observed several positive effects, mostly including skin manifestations of cGVHD.[69,71,72,86-90] A summary of all these studies is presented in Table 3. However, among many of the responding cGVHD patients, rituximab failed to establish a complete recovery. Furthermore, in responding patients, the positive effect of rituximab only applied to specific manifestations of cGVHD, whereas other signs and symptoms seemed unaffected. In addition, there were also patients who were totally unresponsive to treatment with rituximab. This might be due to irreversible damage induced by antibodies. It might also be possible that some cells are not depleted by rituximab, due to the absence or low expression of CD20. Nevertheless, due to the observed positive effects of rituximab and the poor outcome of the

Table 3. Overview of data obtained from studies which have investigated the efficacy of rituximab in cGVHD patients.

Study	*Effect of rituximab*	*Response rate of patients*
Ratanatharathorn *et al.*[87]	Disappearance of cold agglutinin titers and resolution of Raynaud phenomenon, decline in proteinuria, improvement of sclerodermatous changes of thoracic cage with improvement in pulmonary spirogram, resolution of cervical flexion contracture from diffuse scleroderma and healing of lichen planus.	4 out of 8 (50 %)
Canninga-van Dijk *et al.*[72]	Improvement of skin and oral cavity lesions (5 out of 6 patients), recovery liver function (2 out of 5 patients), improvement itching and redness (all 4 patients with lichenoid changes of the skin), nearly full recovery of hair growth (2 patients with alopecia).	5 out of 6 (83 %)
Okamoto *et al.*[71]	Sclerodermatous cGVHD improvement (significant in 2 patients and slightly in 1 patient).	3 out of 3
Cutler *et al.*[88]	Improvement of cutaneous and musculoskeletal manifestations of cGVHD (2 patients with complete response). Significant decrease in DBY (4 out of 4) and UTY titers (3 out of 3).	14 out of 21 (67 %)
Zaja *et al.*[89]	Improvement of cGVHD manifestations of: skin (17 out of 27 patients), mouth (10 out of 21), eyes (6 out of 14), liver (3 out of 12), lung (3 out of 8), joints (4 out of 5), gut (3 out of 4), thrombocytopenia (2 out of 3) and myasthenia gravis (1 out of 1).	25 out of 38 (65 %)
Ratanatharathorn *et al.*[86]	Durable normalization of thrombocytopenia and complete response of oral lichen planus, skin, xerophthalmia.	1 out of 1
Szabolcs *et al.*[69]	In bullous pemphigoid-cGVHD, treatment led to a decrease in bullous pemphigoid titer, however inadequate. Addition of daclizumab was necessary to achieve complete clinical response as well as complete decrease of BP titer.	1 out of 1
Benson Jr *et al.*[90]	Improvement of pure red cell aplasia as a manifestation of cGVHD, as evident from increased reticulocytes and persistent normalization of hemoglobin levels.	1 out of 1

patients who inadequately respond to cyclosporine A and prednisone, rituximab should be given a chance as second-line treatment in these patients, particularly when the skin is involved. However, the question remains how rituximab exerts its effects, as rituximab eliminates $CD20^+$ B cells while the antibody-secreting plasma cells are thought to be $CD20^-$. Perhaps the early B-cell tolerance checkpoints, in which autoreactive B-cells and antibodies are normally removed, are defective in cGVHD, as has been shown to be the case in SLE.[91] This could result in an accumulation of these autoreactive B cells and antibodies in the circulating mature naïve B-cell compartment. These mature naïve B cells may somehow receive signals from the autoreactive B cells and antibodies and subsequently differentiate into plasma cells which secrete autoantibodies that are observed in cGVHD. Rituximab may target these $CD20^+$ mature naïve B cells, which could result in a decreased life span of antibody-secreting plasma cells (Figure 1). However, it is important to realize that plasma cells are not only limited to the bone marrow, as long-lived plasma cells are also found in the spleen and lymph nodes.[92-96] In addition, it was recently demonstrated that the human tonsil contains long lived plasma cells, most of them expressing CD20.[97] These cells were shown to be depleted after treatment with rituximab. Interestingly, in the same study, about 50% of the plasma cells in the spleen, lymph nodes and bone marrow, were also found to express CD20, at a density less than that of plasma cells from blood or tonsil. This suggests that rituximab might exert its effect at these sites. Therefore, the much heard assumption, that plasma cells only reside in the bone marrow and that they are $CD20^-$, has been shown to be incorrect.

Conclusions and future recommendations

Considering the clinical resemblance between cGVHD and autoimmune diseases such as PBC, Sjögren's syndrome and scleroderma,[6,7] in combination with the prevalence of several antibodies (Table 2), a significant role for B cells in the pathogenesis of cGVHD seems plausible. The autoantibodies are probably derived from donor lymphocytes, and are, therefore, in fact alloantibodies, but definite data confirming this assumption is lacking. However, 22 out of 35 antibodies failed to show a strong association with cGVHD, despite their presence (Table 2). Nevertheless, it may very well be possible that these antibodies are allready present before the diagnosis or the onset of the first clinical manifestation of cGVHD. This appeared to be the case in some patients described by Dighiero *et al.*[98] and this phenomenon has also been demonstrated convincingly in SLE.[99] In addition, there might also be an association between these 22 antibodies and cGVHD, when taking the new National

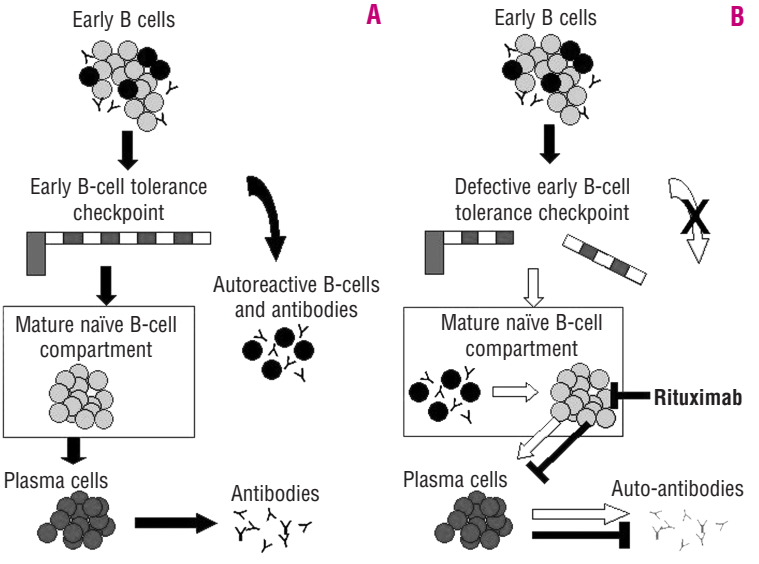

Figure 1. Hypothesis regarding the mechanism of rituximab in cGVHD, based on defective early B-cell tolerance checkpoints. Panel A (left) depicts the cGVHD-negative environment. Panel B (right) depicts the cGVHD-positive environment, with a defective B-cell tolerance checkpoint, eventually leading to the formation of autoantibodies. The inhibitory effect of rituximab on CD20⁺ mature naïve B cells and finally on the formation of autoantibodies is illustrated.

Institutes of Health consensus criteria of cGVHD into consideration.[100-105] The new criteria for diagnosis and staging of cGVHD indicate that there is no longer a time limit after HSCT for the appearance of clinical symptoms of cGVHD,[100] while in the past only the presence or continuation of GVHD manifestions at 100 days or later post-HSCT were termed cGVHD. Importantly, Svegliati *et al.* have demonstrated the presence as well as the biological activity of anti-PDGFR antibodies, leading to fibroblast activation, in sclerodermatous cGVHD patients.[50] Also, the fact that several positive effects of treatment with rituximab in cGVHD patients have been found, further strengthens the involvement of B cells in cGVHD.[69,71,72,86-90] Alternatively, a potential role of B cells as antigen-presenting cells in cGVHD can be considered. Antibodies in the plasma membrane (B-cell antigen receptors) may facilitate the uptake of extracellular antigens via receptor-mediated endocytosis. Eventually, this could result in the cross-presentation of these antigens via MHC class II molecules to T cells.

The complexity of cGVHD is illustrated by the fact that cGVHD is a multi-organ disease and that the manifestations can vary considerably among patients. Thus, it can be hypothesized that the immune system adapts itself to each specific condition predominantly via the use of B cells as well as T cells, since the evidence for dendritic cell involvement is less convincing. For instance, this can be supported by the observations that in some cases of cGVHD there are low levels of $CD4^+$ $CD25^+$ T cells,[56,57,73] whereas in other cases the levels of $CD4^+$ $CD25^+$ T cells are high.[58,69]

Future directions should be aimed at more intensive screening of the autoantibodies and alloantibodies found to date in patients with cGVHD, diagnosed with the new National Institutes of Health consensus criteria, as most studies have analyzed different antibodies (Table 2). It is also important to design more longitudinal studies instead of cross-sectional studies, preferably prospectively, so that changes can be followed over time. Also larger patient groups would be beneficial in order to further strengthen the data. In addition, studies investigating the effect of various therapeutic agents such as methothrexate, cyclosporine A, azathioprine and prednisone, on the kinetics of antibody formation, should be expanded. Furthermore, studies should focus more on identification of novel antigens which might be involved in cGVHD. The importance of such studies is demonstrated by Miklos *et al.*, who concluded that the presence of H-Y alloantibodies appeared to be associated with cGVHD and also with the maintenance of disease remission, in male patients with female donors.[53] Considering the recent finding by Svegliati *et al.*,[50] the role of B cells in the process of fibrosis should be evaluated. Also the possible role of B cells as antigen presenting cells in cGVHD should be investigated.

In conclusion, it is highly probable that both B cells as well as T cells have a crucial role in the pathogenesis of cGVHD. An approach to prevent cGVHD might therefore be to deplete both these B and T cells, followed by infusion of donor Tregs.

Authorship and Disclosures

RK: wrote and designed the paper, and also created all tables and figures. SE: co-wrote and revised the paper. AH: co-wrote and revised the paper, and takes primary responsibility for the paper. AH is advisor to Roche International, Switzerland; Bayer Schering Pharma, Germany and Genmab A/S, Denmark.

The other authors reported no potential conflicts of interest.

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