

Biology of Chronic Graft-versus-host Disease: Implications for a Future Therapeutic Approach

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Abstract

Hematopoietic cell transplantation (HCT) is frequently complicated by graft-versus-host disease (GVHD). During the past three decades, experimental studies and clinical observations have elucidated the pathophysiology of acute GVHD, but the biology of chronic GVHD is much less well understood. Recommendations of the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD have begun to standardize the diagnosis and clinical assessment of the disease. These criteria have emphasized the importance of qualitative differences, as opposed to time of onset after HCT, in making the distinction between acute and chronic GVHD. Experimental studies have generated at least four theories to explain the pathophysiology of chronic GVHD. These theories include 1) thymic damage and defective negative selection of T cells generated from marrow progenitors after HCT, 2) aberrant production of transforming growth factor- β , 3) auto-antibody production, and 4) deficiency of T-regulatory cells. Recent studies in humans have corroborated a possible role for each of these mechanisms in humans. No animal model fully replicates all of the features of chronic GVHD in humans, and it appears likely that multiple biological mechanisms account for the diverse features the disease. Chronic GVHD may represent a “syndrome” with diverse causes among individual patients. In the future, it might become possible to tailor specific therapeutic interventions for patients as individually needed for each distinct pathophysiologic mechanism involved in development of the disease.

Clinical presentation and significance of chronic GVHD

Chronic GVHD is a pleiomorphic syndrome with onset generally occurring between 3 and 24 months after allogeneic hematopoietic cell transplantation (Table 1).¹ The highly variable clinical manifestations of chronic GVHD frequently involve the skin, liver, eyes, mouth, upper respiratory tract, esophagus, and less frequently involve serosal surfaces, lower gastrointestinal tract, female genitalia, and fascia.² The biological mechanisms leading to chronic GVHD are not as well understood as those leading to acute GVHD. Although acute GVHD has been recognized as a risk factor for chronic GVHD, not all cases of acute GVHD evolve into chronic GVHD, and chronic GVHD can develop in the absence of any prior overt acute GVHD. In the skin, the initial phase of chronic GVHD is characterized by an intense mononuclear inflammatory infiltrate with destructive changes at the dermal-epidermal junction, accompanied by irregular acanthosis, hyperkeratosis or atrophy, progressing to dermal fibrosis and sclerosis.³ Other hallmarks include destruction of tubuloalveolar glands and ducts in the skin, salivary and lacrimal glands and respiratory epithelium, and destruction of bile ducts in the liver.

Immune-mediated mechanisms of fibrosis

Insight regarding the pathophysiology and immunobiology of chronic GVHD is limited. A wide variety of experimental models have indicated an association between type-2 polarized immune responses and the development of fibrosis,⁴ and donor type 2 immune responses are required for induction of skin GVHD in mice.⁵ Complement factor 5 (C5) has been identified as a quantitative trait that modifies liver fibrosis in mice and humans,⁶ and C5b-9 complexes are deposited in the skin, liver, lung and kidney in mice with GVHD.⁷ C3 is deposited at the dermal-epidermal junction in humans with chronic GVHD,⁸ but deposition of C5b-9 complexes has not been described.

Animal models of chronic GVHD

At least four different hypotheses regarding the pathogenesis of chronic GVHD have emerged from studies with animal models. One hypothesis posits that chronic GVHD results from thymic damage caused by acute GVHD, resulting in failure to delete nascent T cells that recognize antigens on donor or recipient cells. A second hypothesis implicates a central role for TGF- β in the pathogenesis of the disease. A third hypothesis implicates B cells and antibody-mediated mechanisms in certain manifestations of the disease. Lastly, deficiency in the numbers or function of T-regulatory cells might contribute to the development of chronic GVHD.

The literature is confusing because the term “chronic GVHD” has been used to describe a syndrome of antibody-mediated glomerulonephritis that occurs when recipient B cells are activated by donor CD4 cells after transplantation of spleen cells from certain parental strains into non-irradiated F1 mice. The resulting nephrotic syndrome is more characteristic of lupus nephritis as opposed to chronic GVHD, although case reports have occasionally described nephrotic syndrome in patients with chronic GVHD.

Failure of negative selection in the thymus. Cutaneous changes of acute and chronic GVHD occur in H-2^b MHC-identical transplants between LP and C57BL/6 (B6) mice. Parkman⁹ showed that clones from B6 recipients with chronic GVHD were all CD4⁺ and all showed IL-2-dependent proliferative responses specific for MHC class II I-A^b antigens expressed by both the donor and recipient. The observation that CD4⁺ clones from recipients with chronic GVHD showed specificity for I-A^b suggested that these cells had emerged from marrow progenitors that escaped negative selection in the thymus, and the observation that similar clones could be detected in mice with acute GVHD suggested that the processes responsible for generating such autoimmune clones begin early after transplantation.

Acute GVHD causes severe histopathological damage in the thymus, including injury to medullary epithelial cells, effacement of the corticomedullary junction, disappearance of Hassal's corpuscles and depletion of CD4⁺CD8⁺ cells.¹⁰ In the thymus, developing T cells that express receptors with high affinity for peptide-MHC complexes or "self"-antigens on adjacent cells are deleted. This process of negative selection occurs in the medulla and is mediated by marrow-derived dendritic cells and thymic medullary epithelial cells. Negative thymic selection among T cells developing in mice with GVHD is impaired,¹¹ and the T cells developing in mice with GVHD are pathogenic. Zhang et al.¹² showed that dendritic cells were depleted in the thymus of B6 mice with acute GVHD caused by donor C3H.SW CD8 cells. The resulting thymic damage allowed the development of CD4 cells that responded vigorously to recipient B6 alloantigens. These CD4 cells caused chronic GVHD when they were adoptively transferred into irradiated secondary B6 recipients. Administration of keratinocyte growth factor (KGF) at the time of the transplant enhanced reconstitution of dendritic cells in the thymus, and the CD4 cells that emerged from KGF-treated recipients did not cause GVHD in secondary B6 recipients.¹²

Zhang et al.¹² also showed that donor-derived C3H.SW CD4 cells from B6 recipients with GVHD caused acute hepatic and intestinal GVHD when they were adoptively transferred into irradiated secondary C3H.SW recipients. Further evidence for "autoimmune" recognition among T cells developing in mice with GVHD was reported by Tivol et al.¹³ in experiments where adoptively transferred spleen cells from B6 → BALB/c chimeras with GVHD were adoptively transferred into nonirradiated secondary immunodeficient B6 or BALB/c recipients. Cells from the chimeras caused colitis in secondary B6 recipients but not in BALB/c recipients. In a different approach, several groups have shown that transplantation of MHC-class II-deficient marrow into wild-type recipients of the same strain causes autoimmune damage in the skin,¹⁴ liver and

intestines.¹⁵ Thymectomy prevented the disease, and adoptive transfer of CD4⁺ cells caused acute GVHD in irradiated secondary recipients of the same strain.

In a variation of the same approach, Sakoda et al.¹⁶ found that irradiated C3H (H-2^k) recipients reconstituted with T cell-depleted marrow from MHC-class II-deficient B6 (H-2^b) donors developed a disease with clinical and histopathological features characteristic of chronic GVHD, including epidermal atrophy, follicular dropout, fat loss, dermal fibrosis, bile duct loss, with inflammation, atrophy and fibrosis of acinar tissue in the salivary glands. Thymectomy prevented the disease, and the chimeric CD4 cells proliferated in response to donor-type B6 antigen-presenting cells (APCs) but not in response to recipient-type C3H APCs. Adoptive transfer of chimeric CD4 cells in the presence of B6 APCs caused chronic GVHD in irradiated secondary C3H recipients. In irradiated secondary B6 recipients, however, the adoptively transferred CD4 cells caused acute GVHD.

These results are consistent with those of an earlier study suggesting that CD4 cells cause acute GVHD when they recognize antigens expressed by recipient epithelial tissues but cause chronic GVHD when they recognize antigens on marrow-derived cells but not on epithelial tissues.¹⁷ In these models, the acute or chronic nature of GVHD appears to be dictated respectively by the presence or absence of the recognized antigens on epithelial cells of the recipient. Similar experiments have been done with B6 donors and MHC-mismatched BALB/c or MHC-matched BALB.B recipients. Irradiated secondary BALB/c recipients developed acute GVHD,¹⁸ while secondary BALB.B recipients developed chronic GVHD¹⁹ after adoptive transfer of CD4 cells from chimeric donors with GVHD. Hence, an absence of the recognized antigens on recipient epithelial cells does not entirely explain the development of chronic GVHD.

Further work is needed to define the B6 antigens that stimulate proliferation of donor CD4 cells in the model described by Sakoda et al.¹⁶ In principle, the CD4 cells that develop

in the chimeras are positively selected by thymic cortical epithelial cells of the C3H recipient and negatively selected by MHC class I-positive B6 APCs and by recipient C3H epithelial cells in the thymic medulla. Although the donor-derived CD4 cells that escape MHC-class II-specific negative selection respond to wild type B6 APCs in vitro, the B6-derived APCs in the primary recipients do not express MHC-class II molecules and would not be expected to stimulate donor-derived CD4 cells in vivo. Evidence from at least one study has suggested that the CD4 cells escaping negative selection in the thymus respond to MHC class I antigens.²⁰

Taken together, the experiments with mice demonstrate that acute GVHD impairs negative selection of T cells in the thymus and that CD4 cells recognizing donor or recipient alloantigens can cause a syndrome with remarkable similarity to chronic GVHD. Further work is needed to determine whether these observations have relevance for chronic GVHD in humans. As reported by Tsoi et al.²¹ in 1980, donor-derived T cells from patients showed proliferative responses after stimulation with cells from HLA-identical sibling recipients in 14 of 22 (64%) cases with chronic GVHD and in only 1 of 12 cases without chronic GVHD (Table 2). Responses after stimulation with recipient cells could not be explained by progeny of mature T cells in the graft, since T cells from patients with acute GVHD did not respond after stimulation with recipient cells. The presence of alloreactive T cells in these patients suggests an impairment of negative selection by thymic medullary epithelial cells of the recipient. Responses after stimulation with donor cells were observed 7 of 22 cases (32%) without chronic GVHD and in only 1 of 12 cases without chronic GVHD. In all but one of these 8 cases, responses were also observed after stimulation with recipient cells. These results suggest that the primary defect associated with chronic GVHD in humans is an impairment of negative selection mediated by medullary thymic epithelial cells, with or without concomitant impairment of negative

selection mediated by donor-derived dendritic cells. It seems unlikely that this mechanism can account for all cases, however, since donor-derived cells from 7 of the 22 patients with chronic GVHD showed no measurable proliferative response after stimulation with either recipient or donor cells.

Role of TGF- β . A syndrome characteristic of chronic GVHD develops after transplantation of B10.D2 lymphoid cells into irradiated BALB/c recipients.²² Skin changes include a mononuclear infiltrate deep in the dermis, loss of dermal fat, increased collagen deposition, and “dropout” of dermal appendages such as hair follicles, but in distinction to findings in acute GVHD, apoptosis of basal epithelial cells at the dermal-epidermal junction does not occur. Skin changes begin as early as day 11, and cutaneous fibrosis is apparent as early as day 21. Deposits of IgG, IgA and IgM appear at the dermal epidermal junction in this model.²³ Additional features of the disease in this model include inflammation and fibrosis in salivary and lacrimal glands, sclerosing cholangitis, progressive renal and gastrointestinal fibrosis, and development of anti-Scl-70 antibody.²⁴

Naïve donor CD4 cells initiate the disease in this strain combination,^{25,26} and the dermal infiltrate is comprised of T cells, monocytes and macrophages.²⁷ Antigen presenting cells of either the donor or recipient are sufficient to initiate the disease, and costimulation of donor T cells through CD80 or CD86 on APCs is necessary in order to induce chronic GVHD.²⁸ In this model, costimulation of donor CD4 cells through CD40 on APCs is necessary to induce intestinal disease but not skin disease. T cells and macrophages in the skin express TGF- β 1 but not TGF- β 2 or TGF- β 3 mRNA.²⁹ Microarray analysis also showed upregulated expression of type 1 (interferon- γ) and type 2 (IL-6, IL-10 and IL-13) cytokines, chemokines, and a variety of growth factors and cell adhesion molecules in recipients with chronic GVHD as compared to recipients without chronic

GVHD.³⁰ Administration of a neutralizing antibody against TGF- β prevented or reduced virtually all cutaneous manifestations of chronic GVHD, including cellular infiltration, immune cell activation, thickening and fibrosis.²⁷ In contrast, neutralization of TGF- β by administration of latency-associated peptide prevented thickening and fibrosis, but did not prevent influx of T cells and monocytes or immune cell activation in the skin.³¹

Cutaneous manifestations of chronic GVHD develop after transplantation of B10.D2 spleen cells into MHC-matched (H-2^d) BALB/c recipients but not after transplantation of B10 spleen cells into MHC-matched (H-2^b) BALB.B recipients or after transplantation of B10.BR spleen cells into MHC-matched (H-2^k) BALB.K recipients (Table 3).³² In each of these combinations, the background genes of the donor are of B10 origin, while those of the recipient are of BALB origin, and the combinations differ from each other only in the MHC. These observations were interpreted as indicating that H-2^d MHC class II molecules (I-A^d or I-E^d) could present minor antigen peptides derived from BALB skin to CD4 cells of B10 donors, while H-2^b and H-2^k class II molecules could not. Another explanation for this observation could be related to a polymorphism in the TNF- α gene, which is located in the MHC.³³ Expression of TNF- α is reduced in H-2^d mice as compared to H-2^b or H-2^k mice. Since TNF- α is a potent inhibitor of fibrosis induced by TGF- β , it has been proposed that the fibrosis observed with B10.D2 donors and BALB/c recipients might be related to an inability of cutaneous CD4 cells to produce TNF- α .³⁴

Attempts to prevent or treat chronic GVHD through direct or indirect manipulation of TGF- β may encounter unexpected complexity. For example, Asai et al.³⁵ showed that activated donor NK cells could attenuate the severity of acute GVHD through a TGF- β -dependent mechanism. TGF- β has a non-redundant, essential role in limiting T cell and NK cell responses, and TGF- β -deficient mice develop an early onset lethal autoimmune disease.³⁶ The ability of TGF- β neutralization to prevent or treat chronic GVHD may

depend on the context in which the disease develops. Treatment of B10.D2 donors with G-CSF exacerbates the severity of skin GVHD in sublethally irradiated BALB/c recipients. Donor T cells are required for the development of GVHD, but the severity of cutaneous sclerosis is determined by the non-T cell fraction of grafts from G-CSF-treated donors.³⁷ In this model, neutralization of TGF- β from day 0 – 42 had no effect on manifestations of GVHD in the skin, liver or gastrointestinal tract, but neutralization of TGF- β beginning on day 14 appeared to attenuate progression of the disease to some extent in the skin and gastrointestinal tract, but not the liver.³⁸ Neutralization of TGF- β after transplantation exacerbated acute GVHD by interfering with the regulatory effects of TGF- β on proliferation of donor T cells stimulated by recipient alloantigens. Finally, since TGF- β induces Foxp3 expression and T-regulatory function in CD4⁺CD25⁻ precursors,³⁹ TGF- β neutralization could exacerbate GVHD by interfering with development of T regulatory cells.

The role of TGF- β in human chronic GVHD has not been defined. Results of one study showed elevated serum levels of TGF- β in patients with chronic GVHD as compared to patients without chronic GVHD.⁴⁰ The interpretation of these results is complicated, since assays were carried out not with plasma, but with serum, which contains large amounts of TGF- β released from platelets during clotting. Gene expression studies have shown that increased TGF- β signaling in CD4 cells and CD8 cells was associated with a reduced risk of chronic GVHD in humans.⁴¹ These data might appear to conflict with results from experiments with B10.D2 donors and BALB/c recipients, but 4 of the 5 TGF- β pathway genes that were examined also showed increased expression associated with a decreased risk of acute GVHD. The association of increased TGF- β activity with a reduced

risk of chronic GVHD might result from a decreased risk of acute GVHD, since acute GVHD is a well-recognized risk factor for chronic GVHD.

Established chronic GVHD induced by B10.D2 T cells in BALB/c recipients can be reversed by administration of an agonistic CD137-specific antibody, but administration of this antibody at the time of the transplant exacerbated the severity of acute GVHD.⁴² Administration of agonistic CD137-specific antibody has been used successfully to treat CD4-mediated autoimmune diseases in variety of experimental models. Clinical trials will be needed to determine the safety and efficacy of this approach for treatment of chronic GVHD in humans.

Role of B cells. Deposition of antibody at the junction between the dermis and epidermis has been demonstrated in both murine models²³ and in humans⁸ with chronic GVHD. Both euthymic and athymic BALB/c recipients developed high levels of double strand DNA-specific IgG1 and IgG2a autoantibodies in the serum, cutaneous sclerosis, and glomerulonephritis with proteinuria, beginning within the first 2 weeks after transplantation of spleen cells from DBA/2 (H-2^d) donors.⁴³ Induction of disease required both donor CD4⁺CD25⁻ T cells and donor B cells. In the absence of donor B cells, donor CD4 cells caused acute GVHD without cutaneous sclerosis or glomerulonephritis. The relevance of this model for human chronic GVHD could be questioned, since double-strand DNA-specific autoantibodies, immune complex glomerulonephritis and proteinuria are characteristic of systemic lupus but rarely occur in patients with chronic GVHD.⁴⁴

Several lines of evidence suggest that B cells are likely to have some role in the pathogenesis of chronic GVHD in humans. First, anecdotal experience and phase 2 studies have shown clinical improvement in some patients with chronic GVHD after administration of a CD20-specific antibody.⁴⁵ Second, biomarker studies have shown enhanced CD86 expression after TLR9 stimulation of B cells from patients with chronic

GVHD, as compared to those from controls.⁴⁶ Third, patients with chronic GVHD have high levels of B cell activating factor, which promotes the survival and differentiation of activated B cells.⁴⁷ Finally, agonistic antibodies against platelet-derived growth factor receptor (PDGFR) were detected in serum from each of 22 patients with clinical extensive chronic GVHD but not in serum from any of 17 patients without chronic GVHD.⁴⁸ These antibodies induce tyrosine phosphorylation of PDGFR, accumulation of reactive oxygen species, and stimulation of type 1 collagen gene expression by fibroblasts, through a Ras and ERK1/2-dependent signaling pathway. These results suggest novel approaches for treatment of chronic GVHD, since ligand-induced phosphorylation of the PDGFR is susceptible to inhibition by certain tyrosine-kinase inhibitors, including imatinib.

Role of T regulatory cells. Acute GVHD not only impairs negative selection of T cells in the thymus, as discussed above, but also impairs development of T regulatory cells,¹⁸ which might otherwise prevent the development of chronic GVHD. Anderson et al.⁴⁹ showed that donor or recipient-derived T regulatory cells could prevent GVHD caused by B10.D2 CD4 cells in BALB/c recipients, and Zhang et al.⁴³ similarly showed that donor-derived T regulatory cells could prevent GVHD caused by DBA/2 CD4 cells and B cells in BALB/c recipients. More impressively, Fujita et al.⁵⁰ showed that recipient-derived regulatory dendritic cells cultured in medium containing GM-CSF, IL-10, and TGF- β and then matured by exposure to lipopolysaccharide were not only able to prevent GVHD caused by B10.D2 CD4 cells in BALB/c recipients but were also able to reverse previously established GVHD.

Conflicting results have been reported with respect to the role of T-regulatory cells in preventing the development of chronic GVHD in humans. Results of at least two studies suggested that the presence of increased numbers of T regulatory cells in the blood was associated with a decreased risk of chronic GVHD,^{51,52} but these results were not

confirmed by two other studies.^{53,54} The most persuasive results emerged from a study by Rieger et al.,⁵⁵ who showed that colonic mucosa from patients with acute or chronic GVHD contained very low numbers of T regulatory cells relative to the numbers of CD8 cells, whereas specimens from patients with cytomegalovirus infection or diverticulitis contained much higher numbers of T regulatory cells relative to the numbers of CD8 cells.

Future prospects

The results summarized above raise more questions than answers and leave the impression that no animal model fully replicates all of the features of chronic GVHD in humans, and more importantly, that no single biological mechanism is likely to account for the diverse features of chronic GVHD in humans. In the future, it might become possible to tailor treatment for specific abnormalities that contribute to the disease in each individual patient. The disease is clearly initiated by donor T cells that recognize recipient alloantigens, since the incidence of chronic GVHD can be decreased by exhaustive depletion of T cells from the graft. Evidence from animal models has clearly demonstrated that acute GVHD can cause defects in thymic negative selection, allowing the development of alloreactive T cells that cause chronic GVHD. Preliminary results have suggested that administration of KGF could potentially be used to prevent or treat chronic GVHD that results from this type of mechanism. T cells and B cells can also contribute to pathogenesis of the disease through mechanisms that do not necessarily depend on prior acute GVHD or thymic dysfunction. It seems likely that binding of agonistic antibodies to cell surface molecules other than PDGFR might account for certain manifestations of chronic GVHD. If so, then a range of small molecule inhibitors of intracellular signal transduction could one day be used to treat the disease. More importantly, elucidation of the mechanisms responsible for generating pathogenic autoantibodies could provide a

basis for development of methods to prevent chronic GVHD. Finally, development of methods for ex-vivo expansion of T regulatory cells or for improving the generation, survival and function of these cells in vivo could be used to prevent or reverse chronic GVHD caused by deficits in the numbers or function of T regulatory cells.

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Table 1. Overview of Chronic GVHD

- Pleiomorphic syndrome
 - Resembles autoimmune diseases
 - Onset at 3 – 15 months after transplant
 - 40 – 60% incidence among 3-month survivors
 - Involvement of multiple organs
 - Immune dysfunction with risk of infections
 - 30-50% risk of transplant-related mortality
 - Reduced risk of recurrent malignancy
-

Table 2. Responses of T Cells from patients*

Response after stimulation	Chronic GVHD	
	Yes (n = 22)	No (n = 12)
By donor alone, n (%)	1 (5)	0 (0)
By recipient alone, n (%)	8 (36)	0 (0)
By both, n (%)	6 (27)	1 (8)
Neither, n (%)	7 (32)	0 (0)

*adapted from reference 21

Table 3. GVHD phenotypes, according to differences in MHC

Donor	Recipient	MHC	TNF- α Production	GVHD Phenotype	
				Skin	Systemic
B10.D2	BALB/c	H-2 ^d	Low	Chronic	None
B10.BR	BALB.K	H-2 ^k	High	Modified*	Present
B10	BALB.B	H-2 ^b	High	None	Present
B10 x B10.D2	BALB.B x BALB/c	H-2 ^{b/d}	Intermediate	Chronic	Present

*less severe, especially for hair loss; adapted from reference 32