Graft-Versus-Host Disease: New Directions for a Persistent Problem

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Graft-versus-host disease (GVHD) continues to be a major complication after allogeneic bone marrow transplantation, especially with the increasing use of unrelated and mismatched donors. Recently there has been renewed scientific interest in GVHD because of the increasing appreciation of the complexity of the immune responses seen in GVHD. Two basic aspects of the immune response in GVHD, the immunologic target and the effector mechanisms, are now more completely understood. First, the target of the immune response in GVHD has long been felt to be histocompatibility antigens possessed by the host, but not the donor. Recently, recognition of self antigens in GVHD has been documented, showing that GVHD is more complex than simple alloreactivity. Second, the effector mechanism in GVHD was initially felt to be direct cytotoxicity by alloreactive T cells. It is now recognized that cytokines play a central role in mediating many of the clinical and experimental manifestations of GVHD. The development in these two areas will be reviewed and the implications for clinical transplantation discussed.

GRAFT-VERSUS-HOST DISEASE (GVHD) was originally called "secondary disease" to differentiate it from the radiation sickness and aplasia occurring after total body irradiation (TBI). Animals receiving syngeneic transplants recovered normally. Animals receiving allogeneic transplants developed erythroderma, wasting, diarrhea, and jaundice, and ultimately died of secondary disease. Biopsy of the skin of these animals showed vacuolar alteration of the basilar epidermis and dyskeratotic epithelial cells in the epidermis or hair follicle. As the disease advanced, the vacuoles at the basement membrane progressed to cleft and frank subepidermal bulla formation. In the liver, lymphocytic infiltration and necrosis of the small bile ducts were seen. Crypt necrosis leading to eventual mucosal denudation was seen in the intestinal tracts of affected animals.

Further experimental work showed that F1 hybrid recipients given parental haploidentical marrow developed secondary disease, but parental strain recipients given F1 hybrid marrow did not. These seminal observations led to the conclusion that secondary disease was caused by recognition of host histocompatibility antigens by donor (graft) lymphocytes, hence the name "graft-versus-host disease". Billingham summarized these observations by defining the classical requirements for GVHD. These requirements were: (1) "the graft must contain immunologically competent cells"; (2) "the host must possess important transplantation alloantigens that are lacking the donor graft, so that the host appears foreign to the graft and risk therefore, capable of stimulating it antigenically"; and (3) the host itself must be incapable of mounting an effective immunologic reaction against the graft, at least for sufficient time for the latter to manifest its immunologic capabilities: that is, it must have the security of tenure".

AUTOLOGOUS RECOGNITION

Thus, the immunologic recognition and response seen in GVHD were felt to be caused by histocompatibility differences between the donor and recipient. This classic concept of GVHD as delineated by Billingham accounted for both the GVHD seen after marrow transplantation and in immunoincompetent individuals receiving unirradiated blood products. The manifestation of GVHD in the animals was exactly duplicated in human transplants. This concept also accounted for the therapeutic approaches used for GVHD. The original agents used for prophylaxis and treatment of GVHD were lymphocytotoxic agents (ie, steroids, methotrexate, cyclophosphamide, and antithymocyte globulin) used to destroy cytotoxic T cells. However, there were several cases reported of patients receiving syngeneic or autologous transplants developing clinical GVHD.

In most cases, the GVHD involved the skin only. In a few cases, multiorgan disease with involvement of the liver and/or gut in GVHD was observed. Initially, these cases were attributed to unirradiated blood products or severe infections such as cytomegalovirus mimicking GVHD. Over time, however, a more subtle explanation was appreciated. GVHD in these patients was from recognition of autoantigens. The development of an animal model of autologous GVHD allowed new insights into the complexity of GVHD observed clinically as well as

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Submitted March 5, 1994; accepted June 10, 1994.

Supported in part by Grant Nos. 5P01 CA15396-21, 2R01 CA44783-04, NCI 1RO1 CA54203, and 5R01 AI24319-03 from the National Institutes of Health. G.B.V. is a scholar of the Leukemia Society of America.

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0006-4971/94/8407-0037$3.00/0
the development of immune tolerance after bone marrow transplantation (BMT).

Glazier et al. initially described an animal model of autologous GVHD. Rats given TBI in syngeneic transplants with posttransplant cyclosporin (CsA) would develop a syndrome that clinically and histologically resembles allogeneic GVHD. The possibility of strain drift accounting for this observation was investigated by performing autologous transplants where the animals were allowed to reconstitute from a single shielded limb. In both cases, i.e., animals receiving syngeneic marrow or animals undergoing autologous reconstitution, the animals developed autologous GVHD as shown by erythroderma and histology consistent with GVHD. Initial work has shown that autologous GVHD is directed against an apparent public determinant of the major histocompatibility complex (MHC) class II. Monoclonal antibodies (MoAbs) against class II blocked this recognition (in vitro and in vivo) whereas class I MoAbs were ineffectual. Paradoxically, the cells initially involved in this reaction were CD8⁺, whereas class II-restricted T cells belong to the CD4 subset. However, as the disease progressed, both CD4⁺ and CD8⁺ cells were involved in the reaction.

The concept that autoreactive T cells existed was not new, as shown by the autologous mixed lymphocyte reaction. However, this was the first demonstration of an auto-aggression syndrome caused by the failure of self-recognition of MHC antigens. The requirements for the induction of the syndrome have been elucidated. The thymus was critical for development of this syndrome. Animals who were thymectomized or who had shielded thymuses during TBI failed to develop this syndrome. Second, TBI was very important primarily because of the thymic damage induced and the elimination of a peripheral host resistance mechanism (see below). Third, CsA was also needed to induce this syndrome in the majority of animals. CsA has many complex effects on the thymus. CsA ablates the thymic medulla and depletes reticuloepithelial cells within the cortex. Class II expression is markedly reduced within the medulla. T-cell differentiation is significantly affected by CsA. Thymocytes expressing the α/β-cell receptor and CD4⁺ and CD8⁺ cells are significantly reduced. Immature CD4⁺CD8⁺ and CD4⁺CD8⁺ thymocytes are increased, implying a maturational arrest. These immature cells have been detected in the peripheral circulation. Normal mechanism of clonal deletion of self-reactive T cells are inhibited by CsA, again resulting in release of autoreactive cells into the periphery.

However, this disorder is not as simple as production of an autoreactive cell. For the disease to be expressed, autoreactive cells need to be present and the normal peripheral autoregulatory mechanisms need to be disrupted. Autologous GVHD can be adoptively transferred from animals receiving posttransplant CsA into fresh irradiated recipients. However, if normal splenocytes are added, the secondary recipients do not develop autologous GVHD. Furthermore, animals receiving CsA alone without a transplant can be shown to develop autocytotoxic T cells in increased numbers, which can cause autologous GVHD if the animals remain on CsA for protracted (>6 months) periods of time. Thus, there is both the need for an autocytotoxic cell and disruption of normal regulatory mechanisms for autologous GVHD to become manifest (Fig 1).

More recent work has concentrated on the thymic repertoire involved in autologous GVHD. Interestingly, the same limited repertoire of Vβ 8.5 and Vβ 10 cells that has been implicated in spontaneously occurring or genetically determined autoimmune disorders in the rat have also been implicated in autologous GVHD. Cells bearing these markers are expressed in much higher frequencies in animals with autologous GVHD, suggesting much of this disorder is clonally regulated. Furthermore, depletion of these cells from effector splenocytes inhibits the ability to adoptively transfer this syndrome.

Autologous GVHD may play a role in both acute and chronic GVHD occurring after an allogeneic transplant. The intrathymic and peripheral conditions that result in autologous recognition occur after both autologous and allogeneic BMT. Rats receiving allogeneic MHC mismatched transplants do not develop GVHD while maintained on CsA. Upon CsA withdrawal, a majority of animals will develop the clinical manifestation and histologic manifestations of acute GVHD. To determine if the GVHD in the allogeneic recipient included an autologous GVHD component, cells from animals developing GVHD were adoptively transferred into irradiated recipients of the host and donor strain. If the GVHD seen after withdrawal of CsA was purely autologous, donor strain animals should not develop GVHD. However, if there was an autologous component, animals of both the donor and host strain would be expected to develop GVHD. The results were indeed surprising; both donor and host strains developed GVHD, implicating an autologous component to the GVHD after immunosuppression with CsA. Finally, there is intriguing data to suggest that the chronic GVHD seen after allogeneic transplant may be related to poor/dysfunctional immunologic recovery akin to autologous GVHD. The same Vβ repertoire is expressed in experimental chronic GVHD (without CsA therapy) as in autologous GVHD; suggesting that chronic GVHD includes an autoimmune component. Such a hypothesis is supported by the recent demonstration of class I-specific donor antidonor autoreactive T cells in humans and mice with chronic GVHD. Should this finding of a restricted T-cell repertoire be confirmed in human GVHD (either acute or chronic), one exciting application would be the use of monoclonals restricted to certain V regions to treat GVHD. This should permit more specific therapy with less global immunosuppression.

Autologous GVHD is currently being investigated clinically for potential antitumor effects. Animal models of autologous GVHD have shown that the effector cells of autologous GVHD are capable of recognizing MHC class II-positive tumor cells. Early clinical trials have shown promising results with lower relapse rates in patients developing autologous GVHD. Randomized trials are just starting to verify that there is indeed a benefit to autologous GVHD.

**CYTOKINES**

Originally, GVHD was described as a T-cell-mediated disease. The cellular injury in GVHD was thought to be
Fig 1. Effects on CsA on the T-cell development in the thymus. In the cortex of the thymus, CsA may block (I) positive selection and development of major histocompatibility complex (MHC) restriction. In the medulla, CsA does block (II) clonal deletion of autoreactive cells and the development of autoregulatory T cells. Thus, autoreactive T cells that are normally deleted escape from the thymus (I) into the periphery. Autoregulatory cells that are normally produced (II) by the thymus are inhibited (III) by CsA. Likewise, these autoregulatory cells can be destroyed in the periphery by radiation or cyclophosphamide. For the clinical expression of autologous GVHD, there must be both the presence of autoreactive T cells and the failure of autoregulatory mechanisms.

Cyclosporine - Induced Syngeneic GVHD

Autoreactive T Cells

Peripheral Autoregulatory Mechanisms

CD4+ CD8+

1. MHC Class II Recognition
2. Amplification
3. Tissue Destruction in Permissive Environment (Absence of Regulatory T Cells)

1. Eliminated by Preparative Regimen
2. Failure to Reconstitute During CsA Treatment

The cytokines released start a positive feedback loop that result in the actual disease/destruction in GVHD. The cytokine model of GVHD accounts for many of the observations made about GVHD and has already lead to new lines of therapy. In this model, damage to host tissues during chemotherapy radiotherapy and infections results in release of inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1). These cytokines cause increased MHC expression and upregulate other adhesion molecules. The recognition of recipient/donor differences by alloreactive T cells in the donor graft is increased because of the increase recipient expression of MHC and adhesion molecules. The reactive donor T cells then proliferate and secrete cytokines, most particularly, IL-2. The cytokines then activate additional donor T cells and mononuclear cells. Macrophages are induced to secrete IL-1 and TNFα. The

Caused by cellular infiltration of effector cells into target tissues with resultant destruction. The theory of the destruction seen in GVHD was based on the observation of lymphocytes juxtaposed to dying cells (satellitosis) observed frequently in the skin of patients or animals with GVHD. The obvious conclusion from seeing such a union was that the target cell was being destroyed by the effector cell. Immunohistochemical analysis has shown that many, but not all, of these cells have a phenotype more consistent with a natural killer (NK) cell than with mature T cells. These observations have been confirmed by electron microscopy. This observation lead many investigators to rethink GVHD. A new model of the effector phase of GVHD has been proposed. GVHD is seen as a "cytokine storm." Cytokines are initially released during the preparative regimen and then are perpetuated by T-cell recognition of histocompatibility antigens.
cytokines will stimulate both autoreactive and alloreactive T cells. Patients developing either spontaneous or CsA-induced autologous GVHD do so at lymphocyte recovery, again implicating this cascade. Thus, an inflammatory response is set up that involves multiple cytokines recruiting more cells into the response and damaging more tissue. The resulting cytokine cascade eventually produces the clinical manifestations of the syndrome we call GVHD. Many factors, including gut decontamination, sterile environment, and intravenous Ig, which have been found to decrease the incidence of GVHD, interrupt this cascade.\textsuperscript{8,39} Lymphocyte depletion of a marrow graft may, in part, decrease GVHD simply by temporarily removing the lymphocytes necessary to maintain the cytokine cascade. These observations raise the question of whether delaying the infusion of alloreactive T cells until after the initial cytokine wave (damage from the preparative regimen) is over would prevent GVHD while maintaining a graft-versus-leukemic (GVL) effect. Increased relapse rates after lymphocyte depletion of marrow grafts have largely negated the anti-GVHD benefit of these manipulated marrow grafts. Maintaining the GVL effect while eliminating or reducing GVHD has remained an illusive goal. Recent animal experiments suggest that delayed infusion of lymphocytes may achieve this goal by enter hosting the cytokine cascade.\textsuperscript{40} Delayed infusion of normal donor cells after MHC-matched BMT provides an antileukemia reaction without GVHD.

Even before the cytokine cascade was believed to cause the clinical manifestations of GVHD, IL-2 was considered to be critical in the development of GVHD. IL-2 was appreciated early to be critical in T-cell activation. CsA, an agent that inhibits IL-2 secretion, inhibits experimental GVHD and has been shown to be useful in multiple studies of clinical prophylaxis of GVHD, especially when combined with methotrexate and/or steroids.\textsuperscript{41-44} Much of the early work on the importance in cytokines concentrated on the influence of IL-2. Several recent studies have emphasized the importance of IL-2 in GVHD as an initiating event. Two groups have shown that the precursor frequency of antihost-specific IL-2-producing cells predicts for GVHD in HLA-identical sibling transplants.\textsuperscript{45,47} The emphasis on IL-2 as a pivotal cytokine in GVHD also meant that some of the first trials using anticytokine therapy were directed against IL-2. Herve et al\textsuperscript{48} used an anti-IL-2 receptor MoAb to treat steroid-resistant, established GVHD, and although responses were frequently not sustained, responses were seen in over 80% of patients. Recent work has concentrated on the use of humanized anti-IL-2 receptor monoclonals.\textsuperscript{49}

As mentioned earlier, another line of evidence for the involvement of IL-2 in GVHD is the effectiveness of CsA in experimental and clinical GVHD.\textsuperscript{41-44} CsA inhibits IL-2 production and, at higher concentrations, expression of the IL-2 receptor.\textsuperscript{22}

The important role of TNFα in GVHD has come from two lines of evidence. In experimental models, TNFα was found to be an important mediator of the disease and to be produced in increased amounts. Specifically, TNF levels in animals with GVHD were not increased, although there was a marked increase in mRNA levels suggesting that TNF was being produced in increased amounts in the target organs of GVHD.\textsuperscript{50} TNF has many effects that mimic the effects of GVHD when infused into animals. These include cachexia, running, hematopoietic failure, erythroderma, diarrhea, alveolar damage, and, ultimately, death. Moreover, treatment of animals with anti-TNF antibodies can blunt or prevent many of the findings found in GVHD. Clinical studies have confirmed these results and again emphasized the interactive nature of the multiple events occurring during marrow transplantation with GVHD.\textsuperscript{51} These investigators found that TNF alpha levels were increased in patients with GVHD and in patients with veno-occlusive disease (VOD) of the liver. A clinical study has examined the use of anti-TNFα MoAbs in severe steroid resistant GVHD.\textsuperscript{52} As one would predict by the cytokine cascade model, these responses in these heavily pretreated patients were short lived. Blocking a mediator would only be expected to effect the disease during exposure to the MoAb. Likewise, because multiple cytokines would be released, a multiprong approach using multiple anticytokine agents would be expected to have a more profound influence and potentially induce complete responses.

The appreciation of TNF’s role in GVHD has lead to the study of several agents that may influence TNF production. The most well known of these is the use of pentoxifylline, a xanthine derivative, that downregulates TNFα production in vitro. Unfortunately, the initial pilot data supporting the use of this agent in preventing toxicity from preparative regimens, GVHD, and posttransplant therapy, has not been reproduced in a large randomized study.\textsuperscript{53,54} Recent evidence from direct stimulation of monocytes has shown that thalidomide may also be partially exerting its influences in treatment and prevention of GVHD through inhibition of TNFα.\textsuperscript{55}

Another cytokine to achieve considerable attention recently is IL-1. IL-1 is a cytokine produced by monocytes. Minor histocompatibility antigen different strain transplants have shown that IL-1 may be the critical effector molecule in GVHD.\textsuperscript{56} Although IL-2 mRNA was upregulated only during the first week posttransplant in this model, IL-1 and, to a lesser degree, TNF were found to be increased during GVHD. Inhibition of IL-1 by an interleukin-1 receptor antagonist prevented severe GVHD in this animal model. Because of these observations, a phase I/II trial using an IL-1 receptor antagonist for patients with steroid resistant GVHD is underway.

The role of interferon (IFN) in the cytokine cascade has not clearly been elucidated. The most likely IFN to be involved in GVHD is IFNγ. IFNγ may be induced early during the initial injury because of chemotherapy and/or infection. IFN has been found to be increased in patients and animal models of GVHD.\textsuperscript{57,58} IFNγ may function by upregulation of histocompatibility antigens and induction of release of TNF.\textsuperscript{59} The IFNγ primes macrophages to release TNF after exposure to lipopolysaccharide (LPS). LPS is a potent trigger for induction of experimental GVHD, and endogenous LPS has been detected in the serum of animals with lethal GVHD.\textsuperscript{60} Thus, LPS represents another component in the cascade that results in GVHD and emphasizes the interaction of toxicity from the preparative regimen plus the resultant aplasia and GVHD.
Other cytokines such as IL-6, IL-4, IL-8, and IL-10 have not been thoroughly studied in GVHD. The potential role of these other cytokines as well as the interaction among the cytokines and different T-cell subsets (especially Th1 and Th2) need further delineation at this point. Experimental animal data suggest that the Th2 subpopulation of CD4+ cells results in IL-2 production. Tissue injury results in LPS production that stimulates macrophages to produce many cytokines including IFN, TNF, and IL-1. These cytokines cause upregulation of histocompatibility antigens resulting ultimately in increased autologous and allogeneic recognition and increased T-cell activation. These pathways create positive loops of cytokine production and ultimately a "cytokine storm" of GVHD.

A model can be constructed showing the interactive nature of the cytokines identified to be involved in GVHD. In this model, many of the effects of GVHD are directly caused by the cytokines themselves (TNF being the prime example) or by secondary activation of bystander cells such as NK cells causing much of the cytotoxic damage seen in GVHD.

Our view of GVHD has evolved significantly over the last few years. The targets of immune recognition and the effector mechanisms are much more complex than previously appreciated (Fig 2). However, this very complexity has suggested many new ways of preventing and treating GVHD. These more specific maneuvers are not only likely to lead to better control of GVHD, but less global immunosuppression. These therapies should result in improved survival and allow the increasing use of mismatched/unrelated donors.

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