MINIREVIEW

Immunopathogenic Mechanisms of Posttransfusion Graft-vs-Host Disease (43519B)

D. B. BRUBAKER[1]

Inland Northwest Blood Center and College of Pharmacy, Washington State University, Spokane, Washington 99210

Abstract. **Posttransfusion graft-versus-host disease is a lethal adverse effect of blood transfusions affecting the skin, liver, gastrointestinal tract, and, in addition, the lymphohematopoietic systems. It is a disease that has been described for nearly three decades with well over 400 cases known worldwide; however, the immunopathogenesis has not been fully described. By using murine models, combined with human case reports, the immune mechanism and risk factors are outlined. The models and case reports prove a histocompatibility disparity between donor and recipient. The dose of lymphocytes in the products, types of T lymphocyte subsets in the products, and degree of immune suppression in the host are all factors necessary in the immunopathogenesis of posttransfusion graft-versus-host disease. The mechanism is that of acute, lethal, suppressive immune dysregulation in the host (recipient).** [P.S.E.B.M. 1993, Vol 202]

Transfusions of blood and cellular blood components containing viable lymphocytes can result in posttransfusion graft-versus-host disease (PT-GVHD) in immunocompromised patients (1). However, several recent reports of fatal PT-GVHD, presumably occurring in immunocompetent patients, have raised considerable concern (2–9). This concern has provoked recommendations regarding prevention of posttransfusion graft-versus-host disease by the American Association of Blood Banks (10). However, further accumulation of data reflects possible pathogenic mechanisms which may result in optimum guidelines for preventing PT-GVHD. These mechanisms appear analogous to murine animal models.

There are three principle reasons for researching and studying graft-versus-host disease (GVHD). First GVHD remains a major problem in human bone marrow transplantation. Second, iatrogenic GVHD occurs from transfusion of cellular blood products. Third, GVHD is a unique model in which to study the regulation of the immune system. Various models of GVHD have been studied depending on the scientific questions. The parent into F_1 (P-into-F_1) model may be most appropriate when studying cellular interactions and production of autoimmunity. Cytoreduced (i.e., radiation or chemotherapy) recipient models are frequently used to learn the mechanism of GVHD and its clinical complications related to human disease. In the clinical situation of bone marrow transplantation, the animal models are most analogous to the human situation in terms of donor-recipient pair histocompatibility, cytoreduction agents for the recipient prior to transplantation, and consideration of the complications of both acute and chronic graft-versus-host disease. The disagreements that occur in discussions concerning GVHD are due to the fact that different models may involve different effector mechanisms. However, these various models need not be refuted as is done for bone marrow transplantation, but rather applied as representative models for PT-GVHD. The donor and recipient combination may be identical only at the Class I major histocompatibility complex, or compatible only at the

[1] To whom requests for reprints should be addressed at Spokane & Inland Empire Blood Bank, S. 507 Washington St., Spokane, WA 99210.

P.S.E.B.M. 1993, Vol 202: 122–147

0037-9727/93/2022-0122$3.00/0

Class II histocompatibility antigen system, or may be identical at both Class I and Class II histocompatibility antigens both with and without minor histocompatibility differences.

The present discussion will center on murine models of histocompatible and incompatible GVHD immune regulation mechanisms. These models will be compared for the first time with human posttransfusion graft-versus-host disease reports in the literature in terms of the immunogenetic mechanisms, immunocompetence of the host, and the number of immunocompetent cells in the dose from the donor, as well as other factors.

Experimental Murine GVHD Models Across Major and Minor Histocompatibility Barriers

GVHD is a disease that occurs when T cells and/or natural killer (NK) cells in transplanted donor bone marrow or in cellular blood transfusions respond against major and/or minor histocompatibility antigens on the surface of the recipient cells. This definition does not reflect the complexity of the disease, since the exact nature of the cells that mediate or cause GVHD and the antigens that these cells recognize is still not completely understood; however, a large volume of evidence demonstrates immune response mechanisms in the murine model.

Murine models have shown multiple effects of graft-versus-host disease on the host tissue. All of these models are based on the effect of injected allogeneic or semiallogeneic lymphocytes into hosts that are not competent to react against the donor inoculum. In these models, the host's immune incompetence toward the donor lymphocytes may be due to (i) depletion of the immune response through irradiation or cytoreductive agents in the hosts, (ii) immaturity in the neonatal hosts, or (iii) tolerance in F_1 offspring of the parent donor or another strain.

In the irradiation model, the recipient's marrow stem cells and immune system are destroyed prior to donor stem cell transplantation. This GVHD takes into consideration effects of radiation on recipient tissue and is, therefore, very similar to the clinical bone marrow transplant setting. In contrast to the irradiation model, the P-into-F_1 hybrid GVHD model employs mature parental lymphoid cells that are injected into F_1 mice. In the P-into-F_1 model, the F_1 hybrids can be made immunodeficient by irradiation or by taking advantage of an immature immune system of neonates. Many of these models also use immunocompetent F_1 recipients.

The mouse major histocompatibility antigens (H-2) are codominantly expressed in F_1 mice. *In vivo*, the donor cells are stimulated by the alloantigens expressed on the F_1 host cells. This is lymphocyte dose dependent. When large numbers of donor cells ($>1.0 \times 10^7$) are injected into F_1 adult hosts, a disease similar to GVHD in immunocompromised or irradiated hosts develops. These mice exhibit severe wasting, diarrhea, skin lesions, and early death; however, when fewer donor lymphocytes are injected ($<1 \times 10^7$), the resulting GVHD is rarely fatal. However, these mice do have mild weight loss, various degrees of splenomegaly (11), development of histopathologic lesions in lymphoid and nonlymphoid tissues (12), and immunosuppression (12, 13). Although these symptoms may occur, severe wasting or skin lesions rarely occur. This model best represents immune suppression found in humans who are immunosuppressed by blood transfusions (*e.g.*, kidney transplant recipients). The main target of GVHD in the model with unirradiated hosts occurs in the lymphohematopoietic system. The measurable effects in these mice is immune dysfunction displayed by *in vitro* assays (14). Immune dysfunctions induced by graft-versus-host disease have several different forms dependent upon the genetic disparity between the donor and the host (14–16). Both Class I and Class II disparity of the major histocompatibility complex (MHC) antigen causes an acute suppressive and often times lethal GVHD (17). This form of GVHD reflects profound immune deficiency of all T and B cell functions with attack of the donor cells on the lymphohematopoietic tissues, gastrointestinal system, hepatic tissues, and skin (17). When the disparity involves only the difference in Class II (Ia antigen) loci, the chronic stimulatory form of GVHD develops. This form is characterized by limited engraftment of donor cells within the host lymphohematopoietic system, by a limited T cell functional deficiency, and by polyclonal B cell activation and production of autoantibodies (18, 19). Class I disparities alone are rarely sufficient to produce a GVHD in the unirradiated or immunocompetent P-into-F_1 model (16). Class I disparities alone, in association with disparities at minor histocompatibility loci, or with certain viral infections can cause suppressive GVHD (20). Disparities only at minor histocompatibility loci do not generate GVHD in unirradiated hosts. Each of these forms of GVHD will be examined, first as an animal model for analogies to posttransfusion GVHD in humans, then as mechanisms of posttransfusion GVHD.

Class I and Class II (MHC) Genetic Disparity; Full (H-2) Differences. Since 1973, it has been recognized that the transfer of T cells across the MHC barrier results in severe GVHD (21). The MHC disparity, immune competence of the host, and dose of T cells in the inoculum are significant in the development of GVHD. Not until recently has it been realized that isolated cytotoxic and helper T cell subsets are each capable of causing GVHD (20). As shown in Table I, there are two forms of immune suppression: irradiation of the host and high doses of T cells (subsets) in the

Table I. Murine Models across MHC Barriers to Study GVHD

MHC genetic disparity	Donor	Recipient	Immune suppression[a]	Immune reaction[b] (outcome)
Class I & II	CBA	(CBA × B6)F_1	I (850 rad, 8.5 Gy)	GVHD-IS
Class I & II	C3H	(C3H × DBA/2)F_1	I (850 rad, 8.5 Gy)	GVHD-IS
Class I & II	B6	(CBA × B6)F_1	I (850 rad, 8.5 Gy)	GVHD-IS
Class I & II	DBA/2	(B6 × DBA/2)F_1	HD	IS-GVHD
Class I & II	B6	(B6bm1 × B6bm12)F_1	—	GVH-ID (*in vitro*)
Class I & II	B6	(B6 × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.G	(B10.G × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.D2	(B10.G × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.A	(B10.BR × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.BR	(B10.BR × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.D2	(B10.BR × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.D2	(B10.M × DBA/2)F_1	HD	GVHD-IS
Class I & II	B10.D2	(C57BL/10 × DBA/2)F_1	HD	GVHD-IS
Class I & II	C57BL/10	(C57BL/10 × DBA/2)F_1	HD	GVHD-IS
Class I	B10.A	B10.TL	I (700 rad, 7 Gy)	GVHD-IS
Class I	B10.A	B10.TL	—	NR
Class I	B10.A	(B10.A × B10.TL)F_1	I (800 rad, 8 Gy)	GVHD-IS
Class I	B10.A	(B10.A × B10.TL)F_1	—	NR
Class I	B10.A	B10.BR	I (800 rad, 8 Gy)	GVHD-IS
Class I	B10.A	B10.BR	—	NR
Class I	B6 (bbbb)	(B6 × B6bm1)F_1	I (1000 rad, 10 Gy)	GVHD-IS
Class I	B6 (bbbb)	(B6 × B6bm1)F_1	—	GVH-ID (*in vitro*)
Class I	B6bm1	(B6 × B6bm1)F_1	—	GVH-ID (*in vitro*)
Class I	B10.A	(B10.A × B10.A [2R])F_1	—	NR
Class I	B10.A(2R)	(B10.A × B10.A [2R])F_1	—	NR
Class I with minor MHC disparity	A	(CBA × A)F_1	—	GVHD-IS
Class II	B6	(B6 × B6bm12)F_1	I (1000 rad, 10 Gy)	GVHD-IS
Class II	B6	(B6 × B6bm12)F_1	—	IS-GVHD

[a] I, irradiation; HD, high dose inoculum (>10^7 lymphocytes).
[b] GVHD-IS, GVHD-immune suppression; IS-GVHD, immune stimulation-GVHD; GVH-ID, GVH-induced immune deficiency; NR, no reaction.

donor inoculum. Inoculation of neonatal animals (immunologically immature) with high doses of T cells also readily causes GVHD. The injection of donor bone marrow containing T lymphocytes into irradiated F_1 adult mice (P-into-F_1) results in an acute form of immunosuppressive GVHD with high recipient mortality and a short median survival time of 20 days. When the T cells were purified into subsets, different GVHD reactions occurred. A bone marrow transplant with purified helper T cells induced an acute severe GVHD. On the other hand, purified cytotoxic T cells exhibited significantly slower response to GVHD, survival times being more prolonged, but still exhibited a 100% mortality (22). The relative potency of helper T cells and cytotoxic T cells, however, seems to be dependent on the particular strain combination tested. In the C3H-into (C3H × DBA/2)-F_1 combination, cytotoxic T cells were more potent than helper T cells, but in the B6-into (CBA × B6)-F_1 strain combination, both T subsets responded equivalent (22). In the DBA/2-into (B6 × DBA/2)-F_1 combination, the main T cell population, which engrafts for apparently genetic reasons, is the helper T cells (23). Combined helper and suppressor T cells, however, induce a stronger reaction.

The cell populations required to induce acute suppressive GVHD from Class I and II MHC disparity between donor and host include helper and suppressor T cells and NK cells, all present within the donor inoculum. Helper and suppressor T cell subsets are both required for complete development of immunosuppressive GVHD and host immunodeficiency (14, 21, 24). Depletion of T subsets prevents the development of full immune-suppressive GVHD. Early in GVHD, the helper T cell population is activated, perhaps contributing to the transient lymphoproliferation during the first weeks of GVHD. Subsequently, allosuppressor/killer cells predominate (24).

Donor NK cells may be involved in the pathologic changes of GVHD, but this is still a subject of dispute. The increase in NK activity (e.g., killing of YAC-1 tumor target cells) early in GVHD has been correlated with the development of the histopathologic lesions of the skin and with thymic dysplasia (25, 26). Two studies have been very useful in elucidating the possibility of NK activity in GVHD. One study took NK-deficient beige mutant mice as graft-versus-host donors. The recipient mice failed to develop any immune-suppressive GVHD responses, both immunologically and his-

topathologically (27). Similarly, in another study, *in vivo* depletion of an activated population of donor asialo-GM1-positive cells with NK activity precluded development of severe immune-suppressive GVHD (28). The question remains, however, whether the early GVHD cytotoxic cells with activity on NK targets are classic NK cells or T cells activated by the lymphokine releases that occur in GVHD.

The number of T cells in the inoculum as well as the genetic disparity, is extremely important in the induction of GVHD. Host immune incompetence toward the donor lymphocytes may be due to tolerance in F_1 offspring of the parent donor. In P-into-F_1 hybrid studies, the F_1 host is theoretically tolerant of the donor antigens and cannot reject donor cells, allowing the donor cells to attack the alloantigens on the F_1 host cells. This is totally immunocompetent cell dose dependent from the donor (parent). When less than 6×10^7 immunocompetent cells is injected, there is no fatal GVHD, only some weight loss (14). By *in vitro* measurements, the host has minimal immune suppression (14). These animals have no evidence of GVHD, but do have nonlethal immune suppression. When the F_1 host receives high doses ($>1 \times 10^8$) of immunocompetent cells, they develop a GVHD resembling the lethal immune suppression found in irradiated hosts (29). This is classical acute GVHD represented by skin lesions, diarrhea, severe wasting, and early death. As represented in Table I, this form of GVHD is dependent on genetic disparity between both Class I and Class II antigens. The threshold dose of lymphocytes in the inoculum may vary depending on the different strains used in the Class I and Class II disparate models (17). The variation between strains within the Class I and Class II disparity may cause different types of GVHD responses or reactions, even when the dose of immunocompetent cells in the inoculum is kept constant (30).

The immunosuppressive (acute) GVHD is best shown by the inoculation of C57BL/6 (B6) or C57BL/10 (B10) spleen cells into (B6 × DBA/2)F_1 mice, which results in a type of immunosuppressive GVHD exemplified by profound immunodeficiency, anemia, hypogammaglobulinemia, and the appearance of suppressor cells (31). This is referred to as B6-GVHD. The first six models in Table I concentrate on the helper and cytotoxic T cell subsets in the pathogenesis of GVHD. The B10 models (17) and B6 model (32) have also identified suppressor T cells in the pathogenesis of GVHD. All of the B10 strains (i.e., A, BR, D2, and G) as donor lymphocytes into F_1 hybrids have striking suppressive and cytotoxic effects *in vivo* and *in vitro* (17, 32). In some of these P-into-F_1 models, donor suppressor cells completely inhabited the F_1 hybrid spleens (32). These suppressor cells cause suppression of the bone marrow stem cells and lymphoid system. This allows cytotoxic T cells to destroy GVHD target tissues. The pronounced suppressor and cytotoxic T cells in the B6 and B10 strains is opposite to the DBA/2 strain, which lacks cytotoxic T cells. When all of the B10 strain donor cells in Table I are replaced with DBA/2 donor cells, none of the F_1 hybrids develop GVHD (17). These models prove that H-2 disparity, dose of lymphocytes, and types of T lymphocyte subsets in the inoculum are important. But another factor is the tissue origin of the donor inoculum. Spleen and lymph node cells are no different in initiating GVHD, but bone marrow cells frequently fail to induce GVHD. van Elven *et al.* (17) found that B10.D2 donor lymph node or spleen cells caused GVHD in (B10 × DBA/2)F_1 hybrids, but bone marrow cells failed. Spleen and lymph node cells have the same proportion and subsets of T cells as found in the peripheral circulation. Therefore, the spleen inoculum in animals relates to transfusion-associated GVHD where peripheral blood is used as the inoculum for humans.

Tremendous attention to T cell numbers, subsets, and genetic disparity has been paid to the donor inoculum, but very little attention has been paid to the immune system of the recipient. When B10 mice, which have accentuated cytotoxic/suppressor T cell activity, are crossed with DBA/2 mice, which are depressed in cytotoxic T cells, are the F_1 hybrids normal or does DBA/2 genetics dominate with a F_1 hybrid lacking cytotoxic activity? F_1 hybrids of some strains are more accepting of GVHD than others, which indicates that the genetics in the F_1 hybrid are an important factor (17). How this translates to the immune function of the F_1 hybrids is unclear. Certainly, if the parents have an immune defect, the F_1 hybrid will also. These F_1 mice may appear normal, but function immunologically similar to an intentionally irradiated F_1 hybrid. The reduction of GVHD in neonatal F_1 hybrid mice by inducing tolerance indicates that certain F_1 hybrids have a functional immune system. When (BALB/c × B10)F_1 hybrids were sensitized with either parent's immunologically incompetent T cells, GVHD was reduced (33).

Tolerance in F_1 offspring from the introduction of parental donor T cells to cause GVHD is a complicated model, totally dependent on variables such as donor T cell dosage, subsets of T cells in the inoculum, genetic factors between the parent donor and F_1 hybrid, and the immune competence of the F_1 hybrid.

Class I MHC Genetic Disparity (Class I H-2 Differences). The Class I disparity causes several immunologic responses. Acute, lethal GVHD occurs if the mouse is immunodeficient (by irradiation). A distinction between lethal, immune-suppressive GVHD and GVH-induced immune suppression is an important distinction in Class I disparity. The lethal GVHD has all the clinical symptoms of GVHD, with death occurring in under 100 days. The GVH-induced immune

suppression causes no clinical symptoms in the mouse; they remain alive, but have immune suppression determined by *in vitro* investigations. This latter model is created by transferring parental T lymphocytes into adult, unirradiated F_1 hybrid mice. This leads to immune deficiency of the host, depending on the number of cells injected and the MHC incompatibilities involved.

GVHD only occurs with Class I-restricted donor and host disparity if the host has been irradiated prior to transplantation of hematopoietic stem cells containing T lymphocytes. From previous studies, it has been shown that the T cell subsets which are critically involved in GVHD with Class I disparity are the cytotoxic T cells (20). Purified helper T cells present in the bone marrow transplant were completely ineffective at mediating disease. Similar findings applying to GVHD directed to mutant Class I differences, e.g., with transfer of B6 (bbbb) T cells to mutant Class I (H-2 K)-different bm1 (bm1 bbb) mice. In allelic Class I differences, the lethal GVHD to mutant Class I difference is caused solely by cytotoxic T cells (20).

The injection of parental spleen cells into F_1 mice, whereby the donor T lymphocytes only recognize host Class I antigens as allogeneic, produces GVHD results dependent on strain combinations. In some strain combinations, injection of Class I disparate donor cells has no observable effect. For example, as shown in Table I, injection of (B10.A × B10.A[2R])F_1 mice with either B10.A or B10.A(2R) spleen cells from either parent causes no effect on the host immune function (34). In comparison, if B6 or B6bm1 donor spleen cells are injected into (B6 × B6bm1)F_1 mice, which is GVHD involving recognition of the bm1 mutation, a selective defect in helper T cell function is observed *in vitro* (14). These mice show no clinical evidence of GVHD. Therefore, two different results have been observed in a Class I GVHD: (i) a selective defect in helper T cell function occurs when GVHD involves recognition of the bm1 mutation (14), and (ii) cytotoxic T cells are necessary in the inoculum for initiating and causing GVHD.

Unfortunately, Class I-disparity GVHD is less studied in the murine model and provides less dramatic effects than other MHC disparities. A model probably very similar to Class I and Class II disparities is Class I disparity associated with minor histocompatibility antigens. It produces a severe GVHD. The donor-host combination, caused by injecting A strain donor cells into (CBA × A)F_1 hosts (Table I), differs not only at H-2D, but also at a variety of minor loci (including $M1_5$). This form of GVHD is characterized by T and B cell functional defects, including depressed concanavalin A-induced proliferation, interleukin 1 production, depressed lipopolysaccharide-induced proliferation, and sheep red blood cell plaque-forming cytotoxic responses (35–39). As in acute-lethal GVHD induced by Class I and II disparity, there is an attack on the host stem cell populations, particularly B cells (40).

Class II MHC Genetic Disparity (Class II H-2 Differences). Immunocompetent cells injected into a host with only Class II antigen differences causes three diseases: acute-lethal GVHD, chronic GVHD, and stimulatory (autoimmune) GVHD. The murine model most frequently used is B6-into-(B6 × B6bm12)F_1.

If the host is made immunoincompetent by 1000 rads (10 Gy) of irradiation, acute and chronic suppressive GVHD occurs. The severity of disease is dependent on the dose of helper T cells in the cells injected. The transfer of purified donor B6 helper T cells to irradiated Class II (I-A)-restricted (B6 × B6bm12)F_1 mice causes lethal GVHD directed to the hosts' Class II antigens (20). Recipient mice exposed to heavy irradiation (1000 rad) with low dosages of donor B6 helper T cells (e.g., 10^5 cells) induce an acute-lethal GVHD, with survival less than 21 days. However, lower dosages of helper T cells lead to the development of a more chronic type of GVHD with lower mortality rates. As the dosage of helper T cells increases, e.g., to 2×10^7 cells, most of the recipient mice survive with no symptoms of GVHD or ongoing signs of GVHD. This effect may be similar to the protection seen when high dosages of helper T cells are transferred with cytotoxic T cells to Class I-different hosts (41). With Class II-different hosts, helper T cells are potent mediators of GVHD. At low doses, they allow lethal GVHD; at high doses, they are protective. Whether this is restricted target antigen expression is currently unclear.

The second type of reaction in Class II H-2 differences is immune stimulatory dysregulation. In this form of GVHD, the donor's helper T cells recognize only a disparity in Class II MHC determinants in the host. The result is a chronic immunostimulatory or autoimmune type of GVHD. The degree of T cell immune dysfunction and donor engraftment is limited to helper T cells, compared with the acute-lethal or acute-suppressive GVHD, where the T cell dysfunction is limited to the cytotoxic T cell and natural killer cell with augmentation by helper T cells.

Stimulatory GVHD is generated by at least three different murine models: (i) selection of parental and host strain combinations involving a genetic disparity only at Class II MHC (Class II GVHD as in the B6-into-(B6 × B6bm12)F_1 (18, 21, 42, 43); (ii) depletion of donor LyT-2^+ T cells prior to injection into a Class I plus Class II disparate F_1 host (14, 23); and (iii) injection of DBA/2 donor cells into (B6 × DBA/2)F_1 mice (17–19, 23, 44). All of these combinations result in chronic stimulatory GVHD, as assessed by autoantibody production and selective T cell defects (31). In the first two examples, the donor cells are limited to recognizing Class II MHC by Class II MHC disparity or by reducing cytotoxic cells, which primarily recog-

nized Class I MHC antigens. The stimulatory GVHD in the DBA/2-into-(B6 × DBA/2)F_1 combination was not understood previously because the genetic combination is that of a Class I plus Class II MHC difference, which is similar genetic combinations causes acute-lethal GVHD. Recently, it was discovered that DBA/2 spleens exhibited a 2-fold reduction in cytotoxic T lymphocytes (CTL) and a 9-fold reduction in precursor cytotoxic T lymphocytes, which does not occur in any other Class I plus Class II GVHD (23). Therefore, the DBA/2-into-(B6 × DBA/2)F_1 GVHD resembles a Class II stimulatory GVHD because of the natural deficiency in precursor CTL and CTL for the B6 parent. This is comparable to the example of intentionally depleting CTL in donor cellular inoculum.

There are several distinctions between Class I only, Class I plus II, and Class II only GVHD. Similar to Class I only GVHD, Class II only GVHD is dependent on the degree of donor engraftment. The donor cell requirement is distinctive in each type of GVHD. The main population which engrafts in Class II GVHD is donor helper T cells (14, 23). The T cell chimerism in Class II-different GVHD has been traced by the injection of B6 Thy-1^a lymphocytes, which express the Thy 1.1 allele into (B6 × B6bm12)F_1 hosts. Helper T cells mostly engraft in this combination along with a smaller number of CTL (14). These same results have been observed in DBA/2-into-(B6 × DBA/2)F_1 GVHD (23). The lymphohematopoietic system is not repopulated in Class II disease, unlike Class I plus II GVHD. Stimulatory GVHD is totally dependent on helper T cells, and depletion of CTL does not prevent the development of Class II GVHD as defined by chimerism or T cell functional assay (14).

Minor Histocompatibility Antigen Disparity. Experimental GVHD across minor histocompatibility antigen (MiHA) barriers most resembles the clinical setting of bone marrow transplantation (45, 46). In this setting, the bone marrow donor and the bone marrow recipient are matched at the HLA-A, B, C, and DR loci serologically and to the D region by two-way mixed leukocyte reaction. In many murine systems (Table II), these same conditions apply, i.e., the donors and recipients are MHC identical or MLR nonreactive (45). Currently, there are no typing antisera for MiHA in any animal system. Animal systems of MiHA GVHD are good models for GVHD across minor histocompatibility barriers in humans. It is a good model for bone-marrow-associated GVHD in humans but does not represent PT-GVHD. However, the mechanisms learned from this model may be useful in understanding PT-GVHD.

Most models of MiHA GVHD are understood in mice because of the known transplantation antigens of this species. Nevertheless, there are many variables involved in any given model (47). One model system may differ from another in more ways than the two strains used (47). Factors that influence the severity and outcome of GVHD are: viral infections; microbial flora; whether parental cells are given to F_1 recipients or to another parental strain; the age of the recipient; and whether the recipient is irradiated or chemically immunosuppressed (43). Finally, various assays are used to assess the type and degree of MiHA GVHD. These range from lethality (most crude, but absolute) (45, 46) to subtleties of cellular immune function (48). It is noteworthy that almost all MiHA GVHD models use recipient irradiation (Table II), even P_1 to P_2 models not shown in Table II (49). In the P_1 to P_2 model, irradiation is needed to prevent P_2 anti-P_1 host versus graft (49).

In MiHA GVHD, the distinction between acute and chronic GVHD is somewhat arbitrary. The histopathology of the two are distinct, yet immunologically, there may be a continuum between the two. It is certain that any chronic or *in vitro* MiHA GVHD can be converted to an acute GVHD by changing any number of variables which aggravate the donor-anti-host reactivity (i.e., inoculum dose of T lymphocytes, subtypes, etc.) and by alleviating the host-antidonor reactivity (i.e., immunoincompetence of recipients, age of recipient, etc.). In some strain combinations, such as in B10.BR to CBA mice or vice versa, the transfer of low dosages of T cells (e.g., 10^4 cells) can lead to high mortality associated with a chronic form of GVHD. By increasing the dose of T cells added to the donor

Table II. Murine Models across Minor Histocompatibility Complex Barriers to Study GVHD

Genetic disparity	Donor	Recipient	Immune suppression[a]	Immune reaction (outcome)
None (?MiHA)	B6	LP	I (950–1050 rad, 9.5–10.5 Gy)	A-GVHD → C-GVHD
MiHA	C3H.SW	B6	I (750 rad, 7.5 Gy)	GVHD
MiHA	B10.BR	CBA	I (750 rad, 7.5 Gy)	GVHD
MiHA	DBA/2	B10.02	I (820 rad, 8.2 Gy)	Mild GVHD
MiHA	B10.S	SJL	I (800 rad, 8.0 Gy)	GVHD
MiHA	B10.D2	BALB/c	I (750 rad, 7.5 Gy)	GVHD
MiHA	B10.D2	DBA/2	I (800 rad, 8.0 Gy)	GVHD

[a] I, irradiation.

inoculum, acute GVHD occurs and there is shortened survival of the recipient (46).

The mechanism(s) of MiHA GVHD is most important for bone-marrow-associated GVHD, but may also apply to posttransfusion GVHD. It is useful to know which donor T cells are critical for the induction of GVHD. In MHC GVHD, it is clear that donor helper T cells are most important in GVHD across Class II MHC barriers and that donor cytotoxic T cells are important in GVHD across Class I MHC barriers (49–52). The situation of MiHA GVHD is not clear. T cell subsets are very important in MiHA GVHD. In the B6 to LP model (Table II), cytotoxic T cells are critical (53). In the reverse model, both helper and cytotoxic T cells are needed (54). In the B10.D2 into BALB/c combination, helper T cells are far more important than cytotoxic T cells (55). Whether cytotoxic T cells are more important than helper T cells in MiHA GVHD is unclear. Recent laboratory (56) and clinical (57) evidence supports the fact that cytotoxic T cells and possibly precursors are responsible for MiHA GVHD. However, they also indicate that the presence of helper T cells can accelerate the response. They suggest that the induction of MiHA GVHD may not be limited to the activity of fully mature T cells, but that other immature T cell subtypes, lacking in both the CD-4 and CD-8 markers, may be involved. In line with these observations, van Els *et al.* (58) demonstrated in 16 patients that antihost T cell responses, isolated from the patients' peripheral blood, were significantly higher in patients having acute GVHD than in those having no GVHD. Further studies by the same group found that MiHA GVHD is associated with helper T cells which are restricted by HLA-DR (Class II) molecules (59). This is not unlike the Classes I and II and Class II disparate GVHD indicated earlier.

It is likely that different subsets of donor T cells will mediate different types of tissue damage in the various MiHA GVHD systems. Cytotoxic T cells have been investigated extensively, some appearing to be donor-antihost cells (53, 56, 60), while Parkman has derived autoreactive cytotoxic T cell clones (61). However, in the B10.D2 into BALB/c model, no NK activity has been found (62, 63).

Since cytotoxic and helper T cells express different functions, i.e., lymphocyte-mediated cytolysis and lymphokine production, respectively, the pattern of GVHD may be different for each subset prominence. However, at the population level, both T cell subsets show functional overlap (64–66). Some helper T cells exhibit cytolytic activity (64), while cytotoxic T cell clones release various lymphokines, including interleukin 2 and γ-interferon (65, 66). The primary target organs for GVHD, i.e., the spleen, lymph nodes, liver, and gut, express both Class I and Class II antigens, which provide targets for T cells. Tissue distribution of Class II molecules is limited to a few cells, e.g., macrophages, B cells, and dendritic cells; however, local production of γ-interferon causes up-regulation of Class II expression on epithelial cells (67).

Syngeneic Graft-vs-Host Disease. Several reports have indicated that in humans, a GVHD-like syndrome can occur after marrow transplantation performed between syngeneic twins (identical) (68) or after autologous marrow transplantation (69, 70). These reports have recently been challenged (71) on the general concept that histocompatibility differences between donor and host are absolute requirements for the induction of GVHD, as postulated by Billingham (72). From Billingham's postulates then, what are the inciting responsible antigens and the mechanisms accounting for donor antihost? In bone marrow transplantation, the patient is subject to numerous pharmacologic agents, irradiation, infectious diseases, and environmental insults that may result in perturbation of the immune system leading to a dysregulation of self in the form of non-self-discrimination or autoimmunity. Although GVHD has not occurred in autologous blood transfusions, this animal model is useful in understanding homologous PT-GVHD because many of the pharmacologic agents, irradiation, and viral infections occur in patients who develop PT-GVHD.

A new model was discovered when Glazier *et al.* (73) observed syngeneic GVHD after a combination of irradiation, syngeneic, or autologous bone marrow transplantation and the administration of cyclosporin A (CsA). The pathology observed in this model clinically resembles acute GVHD and is often lethal to a majority of animals. Pathogen-free animals and the animal's age are important factors in the severity of disease (74). After the acute phase, the clinical symptoms may go on to chronicity, similar to allogeneic bone marrow transplantation. As in Class I and II disparate GVHD, syngeneic GVHD can develop a scleroderma-like syndrome in the chronic phase of GVHD (75).

Since this model does not fit with the rules of Billingham as described above (i.e., there appear to be no histocompatibility barriers between donor and recipient), a GVH reaction apparently is not responsible for the disease. Most recently, one proposal (76) chose to revise the rules of Billingham, and another chose to call syngeneic GVHD another term (i.e., bone-marrow-transplantation-associated immune disease) (71). The proposed revisions and terminology revolve around GVHD occurring in MHC indisparity.

In the original rat model, the animals were lethally irradiated and reconstituted with syngeneic bone marrow (73). They were treated with CsA for 40 days and 14–28 days after CsA therapy, they developed GVHD (73). This study does not rule out minor antigen differences due to genetic drift among syngeneic rats as the

target antigens responsible for initiating GVHD. Syngeneic GVHD could be induced in animals undergoing autologous marrow reconstitution after receiving total body irradiation with lead shielding of the tibia. Induction of GVHD by this model indicated that minor histocompatibility antigens were not responsible. This observation indicated to most investigators that histocompatibility differences were absolute requirements for induction of GVHD (72, 73).

Emphasis has been placed on nondisparate histocompatibility as a factor uninvolved in syngeneic GVHD (77). However, Glazier *et al.*'s (73) original studies showed that GVHD could be adoptively transferred by T cells to irradiated secondary recipients. This indicates that x-irradiation must render antigens "unique" in the host for T cell attack and the host must be rendered immune deficient to allow adoptive transmission to occur. The second feature involves immune suppression or dysregulation by CsA. Studies showed that CsA is necessary for the induction of self-reactive T lymphocytes (78). Both irradiation and CsA are required for syngeneic GVHD. Normal, nontransplanted animals treated with very high doses of CsA for short or long periods of time do not develop the autoaggressive disease. Glazier *et al.* (79) found additional evidence that irradiation of the thymus has a synergistic role in causing syngeneic GVHD. Shielding the thymus prevents disease.

CsA has an effect on the thymus. CsA treatment with pharmacologic doses in normal rats and mice induces a rapid ablation of the thymic medulla (80, 81). Only medullary, but not cortical, epithelium was involved (81). One important finding in relation to the thymus and histocompatibility was a markedly reduced expression in Class II major histocompatibility antigens (Ia). Expression of Ia antigens in the cortical area was unaffected. These changes were reversible if the animals were not irradiated; however, these changes were permanent in the irradiated recipient (82).

The initiation of syngeneic GVHD is associated with the development of autoreactive cytotoxic T cells found in the spleen at the onset of disease (83). These cells recognize a public determinant of Ia antigens. The Ia-stimulated cytotoxic T cells are capable of lysing phytohemagglutinin blast cells from several different strains of rats which differed in antigen determinants of the MHC. The blast lysis could only be effectively blocked with monoclonal antibodies recognizing a public determinant to the Ia molecule. In comparison, anti-MHC Class I antibodies were unable to block lysis of blasts (83).

The cellular mechanisms in syngeneic GVHD are similar to MiHA GVHD and both Class I and Class II disparate GVHD. The onset of syngeneic GVHD was associated with the reappearance of helper T cells in the peripheral blood (84), and the disease could be adoptively transferred with helper T cells from animals active with syngeneic GVHD (78). These findings indicated that the generation of IA-autocytotoxic T cells were not the only cells involved in initiation of syngeneic GVHD, but helper T cells were also involved. It is possible that helper T cells provide maturation and amplification signals (i.e., interleukin 2) to cytotoxic T cells (85). Interleukin 2 production is suppressed and affected by CsA; however, this mechanism does not explain the occurrence of GVHD 2 weeks after discontinuing CsA, since interleukin 2 is cleared rapidly. Helper T cells are obviously involved, but it may take 2 weeks before helper T cells become programmed through the thymus to develop (auto) recognition capabilities. The thymus is damaged by irradiation, and may possibly require time to recover.

Histologic evaluation of syngeneic GVHD over time shows that the disease evolves from acute to chronic. During the acute phase, cytotoxic T cells are in the epidermis causing dyskeratosis and injuring epithelial cells (83, 84, 86). In the submucosa and lamina propria of the gut, lymphocytes with both helper and cytotoxic T cell markers are present (87). With progression to chronicity and the development of fibrosis, a number of cells demonstrate coexpression of both helper and suppressor cell markers (88). The majority of infiltrating cells are helper T cells.

In summary, there appear to be few differences between "classical" or bone-marrow-transplant-associated GVHD and syngeneic GVHD. After bone marrow transplantation, stem cells are processed in the thymus to tolerate self (host)-antigens. With irradiation and bone marrow transplantation, donor T cells differentiate in the recipient thymus, but with the occurrence of GVHD, tolerance fails. The host must have target antigens (i.e., minor HA), modified antigens (i.e., drug or irradiation), or autoantigens (i.e., shared donor and host antigens). Damage to the thymus may cause failure to induce tolerance to (auto) antigens in T cells developing from the bone marrow inoculum. This proposes that Billingham's criteria need revision, as proposed by Fischer *et al.* (76) and Bos *et al.* (71). Until there is evidence that there is absolutely no antigen disparity in syngeneic GVHD, the term should be kept to reflect GVHD occurring in autologous or syngeneic human and animal bone marrow transplantation. The major and minor histocompatibility antigen systems are important for transplantation; however, other antigen systems must also be important in transplantation, otherwise we would not have graft rejection or other immune diseases in complete matches.

Role of Irradiation or Cyclophosphamide in GVHD. Irradiation of the host is an important factor in the development of GVHD. Irradiation or cyclophosphamide treatment or a combination renders the host tolerant of the donors' graft (89). In the animal models,

many of the major and minor histocompatibility complex genetic disparities (Tables I and II) require irradiation for the development of clinical GVHD. In genetically disparate animals, irradiation causes immune suppression of the host, allowing donor immune competent cells to attack the host. Large doses of radiation (7.5–12 Gy) destroy the host's capacity to mount an immune response by destroying both unstimulated immunologically competent cells and memory cells (90). Both primary and secondary immune responses are lost (90, 91).

The effect of irradiation on syngeneic GVHD may further explain the effects of irradiation on allogeneic GVHD. In this model, irradiation or cyclophosphamide affected the autoregulatory immune system. This was shown by adoptive transfer of effector splenocytes from animals with active syngeneic GVHD into secondary recipients that were pretreated with various doses of irradiation or cyclophosphamide (92, 93). Successful transfer of syngeneic GVHD only occurred when the secondary recipients received upper or total body irradiation with 7.5 Gy, or were treated with 100 mg/kg of cyclophosphamide. In contrast, syngeneic GVHD could not be transferred into secondary recipients left untreated, treated with low-dose total body irradiation (5.0 Gy), treated with lower body irradiation (10.5 Gy), or treated with busulfan (94). These findings suggest that the thymus is the autoregulatory organ and that it is unaffected by low-dose irradiation.

Role of Cytomegalovirus in GVHD. Cytomegalovirus (CMV) contributes significantly to mortality in allogeneic bone marrow transplantation (95) and it is involved in graft rejection of solid organs (96). Since CMV demonstrates a major role in the immunology of transplantation, it is also of interest in GVHD. Animal models are useful in delineating the role of CMV in GVHD. In the P-into-F_1 model of GVHD, experiments have been performed in which F_1 mice were injected with parental lymphocytes and a nonlethal dose of murine cytomegalovirus. Several methods have been used to study the synergistic effect of CMV on GVHD. In one method, mice were injected with doses of parental spleen cells that were below the threshold for induction of GVHD. Injection of 2.0×10^7 parental cells into an F_1 host induced GVHD, whereas 2×10^6 cells did not. However, the combined effects of murine CMV infection of the F_1 host plus a low dose of parental cells induced GVHD (97, 98). The role of CMV in human GVHD can be extrapolated from the murine model. Sequence homology and immunologic cross-reactivity between an immediate and early antigen of human CMV and the HLA-DRβ-chain were shown (96). CMV-infected cells also produce a glycoprotein homologous to Class I MHC antigens (96). The effect of CMV on the MHC locus may further explain the immunogenetic mechanism of GVHD in humans.

Summary of Animal Models. There are several observations in the animal models that can be applied to human PT-GVHD. In graft-versus-host reactions, the greater the genetic disparity, the more immunosuppressed the recipient for GVHD to occur. The P-into-F_1 hybrid model must have some minor degree of immunosuppression, but not nearly to the same extent as other disparities. The combined Class I and Class II disparate and P-into-F_1 hybrid models both show the most GVHD damage to the animal's immune and hematologic systems, and are the most representative of pathologic findings in PT-GVHD. However, these models have been the only models where effects on the lymphohematopoietic systems were investigated.

The dose of viable immunocompetent cells is also important. Again, the more immunoincompetent the recipient, the fewer immunocompetent cells required in the inoculum to cause GVHD. The P-into-F_1 hybrid model requires very large doses to cause GVHD. The lymphocyte subsets in the inoculum can affect the severity of GVHD.

Cytomegalovirus infections could also be an etiologic factor in PT-GVHD. The animal models clearly indicate this possibility, which has not previously been associated with PT-GVHD.

Pathogenesis of Human Posttransfusion Graft-vs-Host Disease

The pathogenesis of posttransfusion graft-versus-host disease has never been as thoroughly investigated as bone marrow transplant-associated graft-versus-host disease (BMTA-GVHD). All of the PT-GVHD studies have been case reports or surveys. Human PT-GVHD is a lethal disease; therefore, controlled studies cannot be performed. To better understand the pathogenesis of PT-GVHD, we must compile data from the case reports and abstract observations from *in vitro* and *in vivo* animal model investigations that are applicable to PT-GVHD. The animal models are useful in understanding the mechanism(s) of PT-GVHD. In addition, observations from case reports help define immunologic factors involved in PT-GVHD. Several of these factors include HLA typing in reported cases, disease associations and primary diseases, effects of drugs and irradiation, infectious diseases in the recipient, dose of immunocompetent cells, and age of the recipient.

Major Histocompatibility Complex Genetic Disparity. The MHC is a highly polymorphic genetic region on chromosome b that causes significant disparity among individuals. The more disparate the donor-recipient pair are in their Class I and Class II molecules, the more T cells become activated (99). The animal GVHD models have intentionally selected donor-recipient pairs with genetic disparities for both Class I and Class II antigens, Class I antigens alone, and Class II antigens alone. From the previous section, these genetic

disparities all cause acute, suppressive GVHD in the mouse. In humans, all the same Class I and II disparate combinations exist, and the outcome is almost universally acute, lethal, immunosuppressive GVHD.

Table III indicates that the same MHC disparities present in the mouse models (Table I) are also found in human PT-GVHD (6, 8, 100–112). These include disparity in Class I alone (100, 101), disparity in Class II alone (102), both Class I and Class II limited disparity (homozygous haploidentical or P-into-F_1-like) (6, 8, 103–108), and total Class I and Class II disparity (101, 109–112).

There are several problems identified in approaching MHC genetic disparity in human PT-GVHD. First, the number of HLA-typed cases in the literature represents less than 10% of the actual cases. Second, many

Table III. Human MHC Disparity in Cases of Posttransfusion GVHD[a]

MHC genetic disparity	HLA types		Relationship	Recipient diagnosis	Ref.
	Donor	Recipient			
Class I	A3 Ax B35 B38 DR5 DR6	A3 A2 B44 B15 DR5 DR6	Random donor	Hodgkin's disease	100
Class I (2 antigen match)	A29 A28 B12 B18	A29 A1 B12 B7	Random donor	Hodgkin's disease	101
Class II	A2 A24 B15 B15 MLR stimulation index = 4	A2 A24 B15 B15	DD sister	LR renal transplant and non-Hodgkin's lymphoma	102
Class I & II (P-into F_1-like)	A1 AX B8 BX DR3DR- Dw3Dw-	A1 A29 B8 B12 DR3 DR7 Dw3Dw7	Random donor	Lymphoblastic lymphoma	103
	A2 A2[b] B35 B35 DRw11 DRw11 DQw7 DQw7	A2 A29 B35 B14 DRw11 DR7 DQw7 DQw2	DD daughter	Renal carcinoma	104
	A26 A26 B38 B38 DR4w53DR4w53 DQ3 DQ3	A26 NT B38 NT DR4w53 NT DQ3 NT	DD son	Open heart surgery	6
	A24 A24 B35 B35 DR4 DR3 DQ3 DQ2	A24 A28 B35 B44 DR4 DR5 DQ3 DQ3	DD daughter	Open heart surgery	6
	A24 A24 Bw52 Bw52 DR2 DR2 DQw1 DQw1	A24 A2 Bw52 Bw46 DR2 DRw9 DQ1 DQx	Random donor	Cholecystectomy	8
	Aw33 Aw33 B44 B44 DRw13 DRw13 DQw1 DQw1	Aw33 A2 B44 Bw46 DRw13 DRw8 DQw1 DQw1	Random donor	Esophageal cancer	105
	NT	NT	Mother's platelets to infant	Neonatal alloimmune thrombocytopenia	106, 107
	A1 Ax B8 Bx	A1 A28 B8 B35	Mother's platelets to infant	Neonatal alloimmune thrombocytopenia	108
Class I & II	A1 A2 B7Bw44	A25 (10) Ax B18 B38	Random donor	Hodgkin's disease	109
	A3 Ax B7Bw35	A2 NT B40 NT	Random donor	Neuroblastoma	110
	A2 A3 B7 B44	A2 A26 Bw58 B38	Random donor	SCIDS	111
	A3 Aw33 B7 Bw22 Cw2Cw-	A2 Aw24 (9) Bw62(15) B27 Cw3 Cw- DR4 DR4	Random donor	Rhabdomyosarcoma	112
	A2 A3 B22 B12	A9 A1 B5 B15	Random donor	Hodgkin's disease	101

[a] Abbreviations used in table: DD, directed donor; LR, living relative; NT, not tested; SCIDS, severe combined immunodeficiency syndrome.
[b] Probable haplotypes.

of these case reports were HLA typed before Class II antigen typing, and many splits or new antigens have been identified recently. Third, none of the animal models have genetic situations where there is a two-antigen match, much familiar to transplantation immunogenetics. Fourth, there is no true P-into-F_1 hybrids in human PT-GVHD, with one exception, which may remotely occur in the unirradiated mother's platelets (containing viable lymphocytes) to a neonate, diagnosed as neonatal alloimmune thrombocytopenia. Our scope in comparing human case reports with animal models is limited, but useful in understanding the mechanisms of PT-GVHD.

Class I-alone HLA disparity (Table III) is represented by only one case reported by Dinsmore *et al.* (100). There is a one-antigen match at the A locus, but the B locus is totally different. Class II antigens between the donor and recipient appear identical, however, there may be disparity at this locus as well. DR5 has a split between DRw11 and DRw12, and DR6 has a split between DRw13 and DRw14 in which the donor and recipient could be different for Class II antigens. The second report with Class I differences by Burns *et al.* (101) has one patient-recipient pair with a two-antigen match at the A and B loci. There are several reports where Class II typing was not completed and even Class I antigens are incomplete. Incomplete Class I typings result from limited antisera and specificities at the time of typing. The A29B12 haplotype in one of Burns' cases may have been A29B44, since B12 has a split of B44 and B45. B44 has a higher frequency with A29 than B12 or B45.

The case representing the Class II disparate example (Table III) was reported by the author's group; however, the HLA typing was not included in the published report (102). The patient received her sister's kidney and later developed immunoblastic lymphoma. High doses of chemotherapy and irradiation were used to affect the bone marrow. She required two granulocyte transfusions from her sister. From the kidney transplant evaluation Class I antigens were identical; however, there was some variance of Class II antigens (DP), since the mixed lymphocyte reaction (MLR) had a stimulation index of 4. DR typing was not done. After GVHD, the HLA typing was A2A24 B15B15 with an MLR stimulation of 1.0 against her sister's lymphocytes, indicative of PT-GVHD.

Class I and Class II disparities include P-into-F_1 hybrid-like (P-into-F_1-like) examples (Table III). Most of the P-into-F_1-like examples have homozygous haploidentical donors with recipients. There is an exception in Thaler *et al.*'s (6) second case, which is homozygous for Class I antigens but a two-antigen match for Class II antigens. There are no true P-into-F_1-type models because there may be differences in HLA types not identified at this time. In addition, half of these cases were from random donors and, although they appear homozygous for Class I and Class II antigens, they probably are not. With the directed donors, the donation is from children to parents, which is opposite to the animal models (P-into-F_1). Although all of the patients currently appear analogous to the experimental animal model P-into-F_1, other factors must be considered, such as differences in DP antigens and/or minor histocompatibility antigens. The Class II disparity example (Table III) had HLA-matched Class I antigens, and possibly Class II antigens, but the MLR was slightly reactive. The MHC and minor histocompatibility complex antigens in humans have not been fully elucidated. The only actual P-into-F_1 human cases that could be identical to the mouse model are transfusing maternal platelets (containing unirradiated, viable lymphocytes) into infants who have alloimmune thrombocytopenia (due to one of the platelet-specific antibodies, e.g., anti-PI^{A1}) (106–108). In only one of these cases, however, do we have HLA typing to prove PT-GVHD (108). The only means through which this example is identical to the mouse model of P-into-F_1 is if the mother is totally homozygous for HLA Class I, Class II, extended MHC (Class III), and Class IV antigens (molecular-biology-proven homozygosity). Since humans are not an inbred population as are the animal models, the likelihood of this occurring is remote. The one HLA-typed case was: neonate, HLA-A1, A28, B8, B35; mother, HLA-A1, B8; father, A28, A11, B35, B14. The mother was possibly homozygous for A1 and B8; however, family studies and DR and DQ typings were not done, nor were any molecular biology methods. The MLR showed that the infant's cells were unresponsive to its mother's lymphocytes, but were responsive to those of its father and unrelated donors. The stimulation index was not reported, nor was whether the MLR was one-way or two-way stimulation. It is possible that the infant's lymphocytes did not respond to its mother lymphocytes due to engraftment of the mother's lymphocytes in the neonate. The MLR would have been mother's lymphocytes in neonate against mother's actual lymphocytes. Since there are so few cases of PT-GVHD in neonatal alloimmune thrombocytopenia receiving unirradiated platelets, this supports the theoretically rare maternal homozygosity and the fact that these neonates are not immunosuppressed. Additionally, the incidence of neonatal alloimmune thrombocytopenia is 1 in 3000 births requiring maternal platelet transfusions; however, only three cases are reported in the literature of PT-GVHD (106–108).

There are five examples (Table III) where Class I antigens and probably Class II antigens are completely different between donor and recipient (101, 109–112). Unfortunately, none of these cases were completely typed for Class II antigens. Class I and II antigens are frequently linked; therefore, Class II antigens between

the donor and recipient are most likely dissimilar, especially since all the donors are random. The only possibility is dissimilar Class I antigens and similar Class II antigens; however, we only found one case in which this occurred (102). Labotka and Radvany's (112) case is the only Class I and Class II totally disparate example where Class II antigens may have been similar in this group. Failure to type the donor for DR types predisposes this case for a Class I unidentical, Class II similar example.

HLA typing donor-recipient pairs for bone marrow transplantation selection has illustrated the complexity of the MHC loci in finding compatible matches. Understanding of MHC loci complexity in bone marrow transplantation and the occurrence of BMTA-GVHD can be applied to PT-GVHD. Restriction fragment length polymorphism (RFLP) is most useful in determining subtypes DR and DQ, which cannot be determined serologically, for Dw specificities defined previously by homozygous typing cells in the MLR, and for DPw specificities that could only be determined by cellular typing. Al Daccak *et al.* (113) found four groups of matched donor-recipient pairs based on HLA-A,B,DR serology, RFLP for DR, DQ, and DP, by MLR. The groups were: (Group 1) matched pairs with negative MLR, (Group 2) mismatched pairs with positive MLR, (Group 3) matched pairs with positive MLR, and (Group 4) mismatched pairs with negative MLR. Perfect compatibility for DR, DQ, and DP leads to a good clinical outcome. Incompatibility for DP leads to GVHD. Therefore, Groups 2 and 3 had the highest number of acute BMTA-GVHD cases, whereas Groups 1 and 4 had no acute GVHD and good survivals. Linking these observations with the immunogenetics of PT-GVHD, it is entirely possible for a homozygous donor by serologic typing to be dissimilar for DP and for subtypes of DR and DQ. Therefore, what appears to be an HLA homozygous haplotype-identical donor may actually have differences in Class II antigens if RFLP were used, disproving the analogy to the P-into-F_1 mouse model occurring in human PT-GVHD. The other group of interest is the Class I mismatched pairs with negative MLR. These patients do not develop BMTA-GVHD (113). Almost all of the cases from Table III can fit into this group because all PT-GVHD cases were HLA mismatched, but none had an MLR, with the exception of our own case (102).

Despite these observations in HLA typing, the pathogenesis of PT-GVHD is considered analogous to the P-into-F_1 animal model (114–116). Although currently there are proponents of the extended haplotypes, which they consider to be syngeneic MHC matches, molecular biology has disproved linkage between Class III and Class II antigens. Kruskall *et al.* (114) presumes that if the HLA typing is extended to the complement genes, a person homozygous for an extended haplotype can be considered analogous to the inbred P-into-F_1 mouse models. The MHC extended haplotypes include genes other than HLA located between the HLA-B locus and HLA-DR, DQ. These genes include four serum complement protein loci, two loci for 21-hydroxylase, and a locus for the glyoxalase I red cell enzyme (117) Awden *et al.* (118) found that the reactivity in the mixed lymphocyte reaction between unrelated, extended, haplotype-matched individuals was as low as that between identical siblings. However, RFLP typing is refuting this possibility (113).

There is a paucity of PT-GVHD reports in which molecular biology was used. A report by Drobyski *et al.* (119) proved that transfused, irradiated (2,000 rad, 20 Gy) blood products engrafted to cause PT-GVHD in a bone marrow transplant patient. RFLP analysis of the marrow and peripheral-blood cells at the time of graft rejection demonstrated a persistent pattern that was neither the donor nor host, which is indicative of third-party transfusion-associated engraftment. Both polymerase chain reaction and oligonucleotide hybridization proved this. Since the number of lymphocytes were limited, serologic testing for HLA types were not performed (119). Funkhouser *et al.* (120) reported a case of PT-GVHD in a premature infant using RFLP. One (perhaps two) of the four donors engrafted, causing PT-GVHD. Antithymocyte globulin and steroids eliminated at least one foreign allele, but the neonate died (120). The observed incidence of PT-GVHD versus predicted chance of transfusing homozygous donor to heterozygous individual does not match. Kruskall *et al.* (114) estimated that with nine extended homozygous HLA Caucasian random donors, 1 in 500 transfusions should cause PT-GVHD. We have not observed this, but her most frequent HLA homozygous type HLA-A1 B8, SCO1, DR3 was reported in the literature (Table III) (103). We observed in our own transfusion setting a frequency of 1 in 10,000 transfusions (1, 121). Since the Japanese have high homozygous frequencies, one would expect more random donor PT-GVHD implicated from homozygous donors. The predicted versus the observed is variable between reports. For example, the common Japanese haplotype A24-CBL-Bw52-DR2-DRw52-DQw1 has been implicated in two cases of PT-GVHD, and in another case the donor's haplotype was Aw33-CBL-B44-DRw13-DQw1, found in 5.8% of the Japanese population (105). The risk at which a heterozygous recipient would receive homozygous haploidentical blood was predicted in about 1 in 600–700 (7) to 1 in 3,000 (105) transfusions. However, Ino *et al.* (2) reported an actual incidence of 1 in 300–400 transfusions in a survey of open-heart surgery patients. Some transfused patients may be at greater risk, such as multiple-transfusion patients (e.g., with open-heart surgery, trauma surgery, vascular surgery, etc., where the risk is increased as high as 1 log).

In Table III, we have not included two case reports that performed HLA typing because they are incomplete in typing, which causes misinterpretation of results (122, 123). For example, a case by Parkman *et al.* (122) was interpreted by Kruskall (117) as the donor being homozygous for B12. However, all of the donors were incomplete in typing (i.e., Donor 1, A3A2?B7; Donor 2, A1A2?B12; Donor 3, A2/B12; Donor 4, A11A28B12Bx). Transfusions from Donor 4 caused PT-GVHD by cytogenetic studies and may have homozygosity of B12, but the HLA typing sera at that time had multiple specificities. As an example, serum from a donor named Storm was considered specific for H-LA3, but later it was shown to contain A11.

The two cases by Siimes and Koskimies (123) were also incomplete in HLA typing; they missed donor HLA typing. They proved GVHD by family typing and finding HLA types in the bone marrow cells that did not exist in any of the paternal haplotypes. They also found true chimerism in establishing a clone of donor cells in the bone marrow and the patients' type in the peripheral blood. These patients were described as having chronic GVHD. Findings such as these would be more consistent with chronic GVHD than acute PT-GVHD (123).

In summary, most of the different MHC disparate PT-GVHD found in humans have representative animal models. The differences are the P-into-F_1 model, minor MHC model, syngeneic model, and Class II stimulatory model. We observed many P-into-F_1-like cases in humans which best fit the P-into-F_1 animal model.

Immune Mechanisms of PT-GVHD. The cellular and cytokine processes involved in GVHD represent a network of interactions (Figs. 1–4). T lymphocytes play a central role in preventing GVHD, as shown in allogeneic bone marrow transplantation where the T lymphocytes were depleted from the donor's bone marrow transplant (124, 125). The lymphocyte of ancillary importance is the NK cell (126). In the past, cytotoxic T cells and NK cells from the donor were thought to directly attack epithelial cells in the host (127). More emphasis is now placed on the cytokine pathways as the cause of GVHD (115).

The effector cell and cytokine pathways and the target cell damage are similar in Figures 1 through 4. These pathways are synthesized from the previously noted animal models. The initiation process is distinctive for MHC-restrictive stimulation in PT-GVHD. The recipient epithelial cell and bone marrow stem cell are the target cells in PT-GVHD. CTL and NK cells are the main effector cells in PT-GVHD, with helper T lymphocytes and CTL having a role in initiating the network based on MHC restriction. Work taken from the previous mouse models (Table I) is incorporated into each of the Figures. The cytokine-activated T lymphocytes which produce interleukin 2 and γ-interferon are essential to the pathogenesis of PT-GVHD. The potentiation of GVHD by T cell cytokine, interleukin (IL) 2 (128), and the suppression of GVHD by antibodies to the IL-2 receptor indicate that clonal amplification of T cells occurs in GVHD (129). Activated T lymphocytes, both helper and cytotoxic T cells, produce IL-2, which expands the graft-versus-host-reactive effector cell populations to become target cell directed (130). IL-2 continually reactivates the activated donor T cells and clonally expands CTL and NK cells (130). γ-Interferon has an enhancement effect in GVHD (131). Interferons in general heighten expression of cell surface molecules (antigens), particularly MHC molecules. All three classes of interferon enhance the expression of Class I antigens on certain cell populations (132). IFN-γ increases synthesis and expression of Class II molecules on monocytes, macrophages, Langerhans'-γ cells, epithelial cells, and progenitor cells (133). IFN-γ develops IL-2 receptors on CTL and activated T lymphocytes (134). The Fc-γ receptor is expressed on T cells, mainly the CTL IgG receptor (135). This process organizes the CTL to participate in eliminating the donor host's foreign MHC-bearing cells.

IFN-γ is a potent stimulant of NK cells, especially converting precursor NK cells to mature, activated NK cells (136). Pre-NK cells are monocytolytic cells; when activated, they become lytic (136). The NK cell impact in GVHD is less known than the CTL; however, NK cells in the murine models demonstrated a major role in the pathogenesis of GVHD (27). Antibodies to NK cells suppress GVHD in mice (137). In human allogeneic bone marrow transplants, NK cells are detected in the peripheral blood early in GVHD (138).

Direct cell contact (although we include it in the Figures) is not necessary for cytolysis of target cells in GVHD, even though at least 25% of necrotic epithelial or bone marrow stem cells have satellite lymphocytes. Cytokines released by lymphocytes are what cause cell death. Tumor necrosis factor (TNF)-α and lymphotoxin (TNF-β) are secreted by CTL and NK cells, which are molecules capable of forming pores or channels in target cells for lysis of these cells (139). Recently, it was shown that TNF does not cause cellular death (apoptosis) by direct cell necrosis, but rather by a process that involves DNA fragmentation (140). This explains cell death when lymphocytes are not directly adjacent to the lytic cells and why rapidly dividing cells (e.g., bone marrow stem cells, symphoid cells, gastrointestinal cells, and skin epithelial cells) are most affected in PT-GVHD. GVHD can also be suppressed by antibodies to tumor necrosis factor (anti-TNF), which suggests that TNF is the main cytokine responsible for tissue damage (130).

Two common features of target cell attack by lymphocytes in GVHD are the ability to express HLA Class

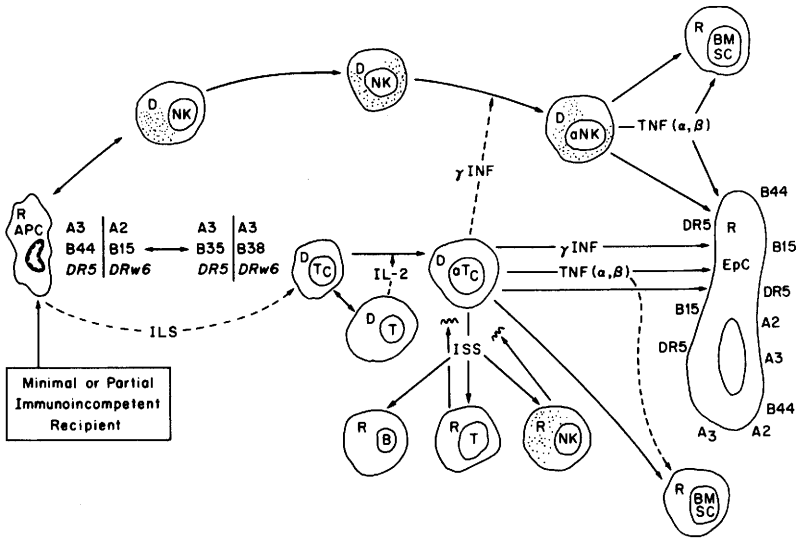

Figure 1. The immune regulatory network of Class I disparity reported by Dinsmore *et al.* (100). In this network, NK cells and cytotoxic T cells (Tc) from the donor (D) interact with the recipient (R) antigen-processing cells (APC) to initiate activation of donor cells for attack on recipient epithelial cells (EpC) and bone marrow stem cells (BMSC). Various cytokines are involved in lysis of the target cells; TNF-α, TNF-β, and IFN-γ. Immune suppressive substance suppresses the recipients immune system, allowing donor cells to attack target tissues.

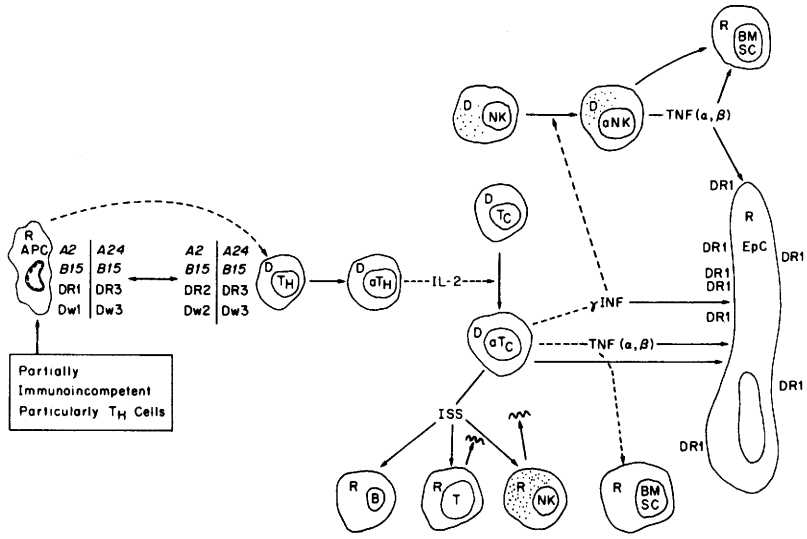

Figure 2. The immune regulatory network of Class II disparate PT-GVHD (102). Here, the helper T cells (T_H) from the donor become activated and initiate target cell attack on the recipient.

I and Class II antigens, in particular HLA-DR, and a high cell turnover (stem cells). Smooth muscle cells, nervous system cells, connective tissue cells, and other cells without or with low HLA-DR expression are not targeted for GVHD. Slow replicating cells also are unaffected. Viral determinant antigens expressed on target cells may increase sensitivity to GVHD attack (141). Bacterial microflora of the skin and gastrointestinal tract may also lead to target cell attack, since antibacterial decontamination diminished GVHD (142). Dividing target cells in the gastrointestinal tract may be damaged by bacterial endotoxin or by antibodies to antigens cross-reactive with the bacteria (142).

Immune suppression of the host is also a key pathway in PT-GVHD. Since this immunopathogenetic mechanism is not well understood in GVHD, we must again rely on animal models. In murine models, the lymphohematopoietic system is mostly suppressed. The bone marrow is virtually decimated in certain strain models (17). In the B6-into-(B6C3)F_1 model, at 6 weeks of GVHD, the hematopoietic stem cell populations in the bone marrow are diminished (143). The lymphoid system is affected by diminution in size of the lymph nodes, spleen, and thymus. During Weeks 3–6, there is an absolute numerical deficiency in T cells in the GVHD mice (144). Primary B cell responses are also defective during GVHD. Lipopolysaccharide response is decreased and plaque-forming cell response

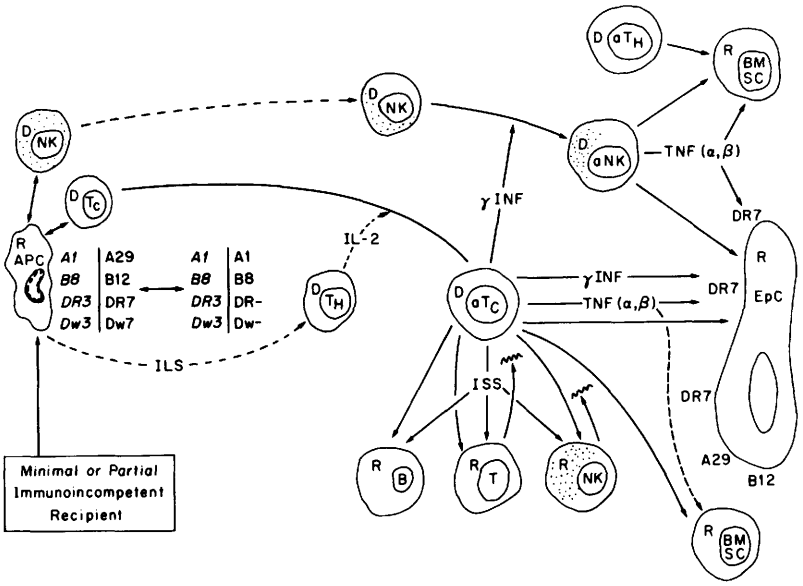

Figure 3. Class I and Class II disparate parent-into-F_1 hybrid-like PT-GVHD. Here the three cells necessary to initiate attack on the recipient's target tissues are NK cells, cytotoxic T cells (Tc), and helper T cells (T_H) from the donor. This example is reported by Weiden *et al.* (103).

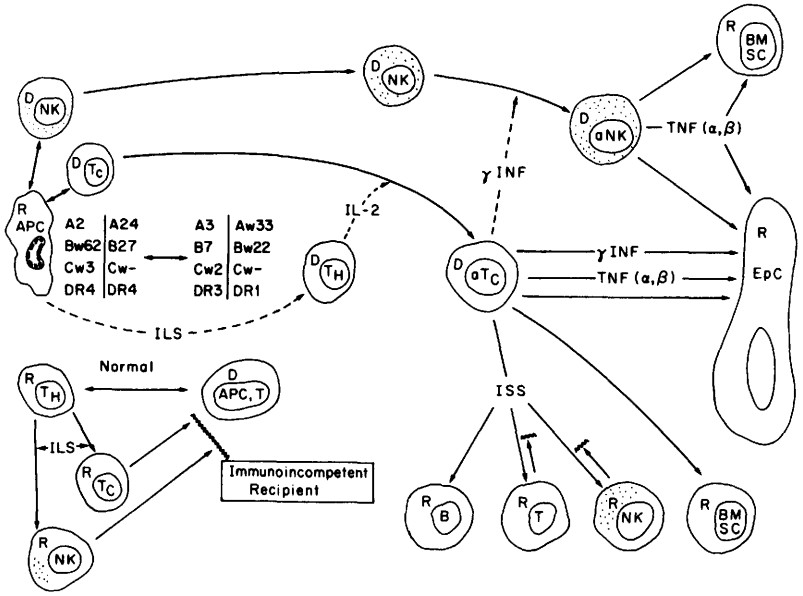

Figure 4. The immune regulatory network of Class I and Class II disparate PT-GVHD. In normal individuals, the recipient's T cells attack foreign donor cells, but if the recipient is immunoincompetent, this is blocked, allowing donor T cells to become activated and attack the recipient's target tissues. This example of HLA disparity was reported by Labotka and Radvany (112).

to sheep red blood cells is depressed for several weeks. Pre-B cells in the bone marrow and B cells in the spleen are deficient in number (17, 143). The spleen is also suppressed. When spleen cells from GVHD spleens are cocultured with spleens from normal host (F_1) or donor strain mice, the immune responses of the normal cells are suppressed. Generation of CTL against allogenic or TNP-modified syngeneic stimulators is suppressed as early as the first week of GVHD (145).

The most important GVHD-induced defect involves thymic function. Both animal models and case reports of PT-GVHD explain the thymic defect. In the acute-suppressive GVHD induced by injection of parental cells into F_1, there is a dramatic effect on the F_1 thymus. Alterations in the thymus from GVHD, much like the bone marrow, permanently compromises the thymus and prevents the ability of the thymus to educate new T cells. During the first weeks of GVHD, the host thymus undergoes marked histologic changes, including a marked decrease in size, loss of thymic epithelium, and Hassall's corpuscles (146). Intrathymic helper and cytotoxic T cells are reduced (147). GVHD

induces thymic hormonal dysfunction (148). GVHD-induced thymic defects are not seen in all strain combinations. BMTA-GVHD in mice in which GVHD was induced by B10.A-into (B10 × B10.A)-F_1 resulted in failure of all T cell responses (149), whereas B6-into (B6 × C3)-F_1 resulted in selective helper cell, but not cytotoxic, T cell dysfunction (150). Whether this mechanism is autorestrictive or allorestrictive (Class I, Class II, or both) is not clear. In the first model (B10.A strain), there is initial suppression of T cell function, depletion of all T cell populations, and no recovery of donor T cells. The second model (B6 strain) has the initial events occur, but donor T cells engraft with recovery of T cell function, indicating thymic function and modification to accept donor T cells. It is apparent that these various effect of GVHD on the thymus in mice is a result of strain combinations (histocompatibility locus antigens), dose of lymphocytes in the inoculum, surviving host lymphocytes, or secondary events (e.g., irradiation, infections, etc). All of these same factors exist in human PT-GVHD.

In humans, GVHD is similar to some of the animal models studied (146). Seemayer and Bolande (151) reported a case of PT-GVHD in a premature infant who developed thymic involution with GVHD that resembled thymic aplasia (151). Unlike thymic aplasia, the GVHD thymus had lymphocyte invasion of the medullary epithelial cells and calcified remnants of Hassall's corpuscles. The medullary epithelial cells showed balloon or exploding cell degeneration and necrosis similar to that seen in the skin, liver, and gastrointestinal tract.

The cause of suppression of the lymphohematopoietic system is unclear, but it is the most understudied area of GVHD. In the B10.D2-into-BALB/c minor histocompatibility disparate model (Table II), suppressor cells were identified in the spleens of BALB/c mice (152). Characterization of these cells showed no mature T cells, NK cells, B cells, or macrophages. Since the cells most closely resembled NK cells, they were named natural suppressor (NS) cells (153). NS cells do not bear phenotypic markers of mature T cells; therefore, NS cells arise from a non-T, non-B cell population (possibly stem cells). The NS cell probably requires T cell signals (e.g., IL-2, IFN-γ), which are generated from donor T cells reacting with the recipient's MHC antigens. Recent evidence suggests that a soluble factor is involved in suppression of the host (154). This soluble substance (Figs. 1–4) is called immune suppressor substance; it suppresses the recipients immune cells (i.e., T, B, and NK). In the murine model, NS cells were identified; however, in humans, the suppressor cell is linked to cytotoxic T cells hypothetically shown producing immune suppressor substance. Theoretically, immune suppressor substance and NS cells in PT-GVHD would prevent the host from reacting and destroying the graft (transfused immunocompetent cells).

The initiation and proliferation pathway in PT-GVHD is similar to the animal model represented by different MHC disparities between donor and recipient, but unique in that the pathology is virtually acute-lethal, immunosuppressive GVHD (Figs. 1–4).

Class I alone disparity (Fig. 1) does not cause GVHD in any of the mouse models (Table I) unless the recipient is immune compromised by irradiation. Some strains are apparently more weak genetically in their immune response and more vulnerable to GVHD, but most all Class I disparate strains are able to reject donor lymphocytes (34). The cytotoxic T lymphocytes in the mouse models are responsible for Class I disparate acute, immunosuppressive GVHD (20).

There is only one representative case of Class I disparate PT-GVHD (Table III) (100). The initiation pathway shown in Figure 1 represents donor cytotoxic T cells and NK cells directly instituting the effector network through Class I differences. Since DR is identical, the requirement for helper cells to introduce CTL and NK cell activation is unnecessary. In the animal models, helper T cells were not necessary for the graft-versus-host reaction (20). Host immune incompetence is also important for GVHD to occur in this example (Table I). The case by Dinsmore *et al.* (100) supports the animal model through immunosuppressive therapy (irradiation and chemotherapy) and by a disease that is characterized by immunodysfunction (Table IV). In this example, CTL and NK cells, along with the cytolytic cytokines, cause target cell death.

Class II disparate PT-GVHD is also limited in examples. The only possible example is the case we reported (102). The patient received high doses of lymphocytes from a sibling with identical Class I antigens and a mildly stimulated MLR. In Figure 2, we include theoretical Class II antigens; nevertheless, there were some slight differences in Class II by the MLR results. In this example, the helper T cells would introduce an activation process through the Class II different interactions between donor and recipient cells. The donor helper T cells would activate CTL, suppressing the recipient's lymphohematopoietic system and activating the effector pathway to attack target cells. Although acute-lethal GVHD occurs in the mouse model, the host must be immune incompetent (by irradiation) before acute or chronic GVHD occurs in these animals. In the PT-GVHD example, the patient received aggressive chemotherapy and had evidence of immune incompetence with a peripheral blood lymphocyte count of less than 100 (Table IV). An immune stimulatory GVHD has not been reported in humans from blood transfusions. Perhaps the exact genetic disparity that occurs in the mouse to cause this disease cannot be duplicated in humans.

Table IV. Immunopathogenic Factors in PT-GVHD[a]

Diagnosis	Cytoreduction	Lymphocytopenia[a]	Age	Infectious disease	Recipient immune status	Lymphocyte dose	Ref.
HD-MC stage IIIA	Five cycles of MOPP & ABDV and 2000 rad	No, 1.5×10^9/liter, E	18	0	Depressed (probable)	$2–5 \times 10^9$	100
HD-NS stage IVB	MOPP & radiation	NR	37	NR	Depressed (probable)	3.5×10^9	101
HD-NS stage IIA	4400 rad and five cycles MOPP	0.23×10^9/liter	21	?	Depressed	3×10^8	101
NHL	Cytoxan, doxorubicin, vincristine, prednisone	$<0.1 \times 10^9$/liter	30	Possible	Depressed	5×10^{10}	102
NHL	Cytoxan, prednisone, vincristine, methotrexate	$<0.1 \times 10^9$/liter, E	18	Possible	Depressed	5×10^{10}	103
Renal carcinoma and surgery	None	$<0.7 \times 10^9$/liter, E	72	NR	Depressed	2×10^{10}	104
Open-heart surgery	None	No, 4.0×10^9/liter, E	69	NR	Depressed (possible)	6×10^9	6
Open-heart surgery	None	No, 1.35×10^9/liter	51	NR	Depressed (possible)	6×10^9	6
Cholecystectomy	None	$0.8–1.3 \times 10^9$/liter, E	52	NR	Depressed (possible)	9×10^9	8
Esophageal cancer and surgery	None	No, 1.78×10^9/liter	53	NR	Depressed (possible)	9×10^9	105
HD-NS stage III	MOPP	0.36×10^9/liter	58	?	Depressed	6×10^9	109
Neuroblastoma	Cytoxan, DTIC, vincristine	NR	2	NR	Depressed (probable)	6×10^9	110
SCID	None	2.3×10^9/liter	4 mo	Yes	Depressed	9×10^9	111
Rhabdomyosarcoma	Cytoxan, vincristine, doxorubicin, dactinomycin, 5500 rad, 3000 rad	$<0.5 \times 10^9$/liter, E	9	NR	Depressed	6.5×10^9	112

[a] Abbreviations used in this table: HD-MC, Hodgkin's disease-mixed cellularity; HD-NS, Hodgkin's disease-nodular sclerosis; NHL, non-Hodgkin's lymphoma; E, estimate; NR, not reported; SCID, severe combined immunodeficiency.

The genetic disparity currently in favor in transfusion medicine is the Class I and Class II MHC genetic difference. This is especially of interest in the homozygous haploidentical match between donor and recipient (2–9). These clinical cases were considered analogous to the P-into-F_1 hybrid mouse models (114). Although we have no true P-into-F_1 situation in humans, the mechanism of PT-GVHD in humans probably best correlates with the animal models for full H-2 differences.

The cells involved in initiating and proliferating both Class I and II disparate GVHD are NK, CTL, and helper T cells, since the alloantigenic compositions of the recipient and donor are different for Class I and Class II molecules. In mouse models of both Class I and Class II disparate GVHD, helper T cells respond to Class II antigen differences and NK and CTL cells respond to Class I antigen differences. Thus, all three cells initiate GVHD in this situation. The case by Welden *et al.* (103) (Fig. 3) demonstrates the pathogenetic mechanism. Figure 4 exhibits a similar network in initiating GVHD; however, in this example, there is full HLA Class I and Class II disparity with no haplotype matches. This example by Labotka and Radvany (112) probably represents the majority of cases of PT-GVHD, especially since all have some degree of immune incompetence. Figure 4 indicates that in normal or near-normal recipient immune systems, the recipient rejects transfused immunocompetent cells. This occurs by donor antigen-processing cells and T cell activation of recipient helper T cells which stimulate recipient CTL and NK cells to eliminate donor immunocompetent cells. In the Class I and Class II disparate model, the function of all three lymphocytes is important in PT-GVHD (115).

Immunologic Factors to Consider in PT-GVHD

The paramount elements in determining the pathogenesis of PT-GVHD are MHC genetic disparity, degree of immunocompetence of the recipient, and dose of immunocompetent cells. Prior to 1988–1989, PT-GVHD was found only in immunocompromised recipients (1, 155–157). This fit one of Billingham's three criteria for GVHD: the recipient is incapable of reject-

ing the donor cells. The third criterion has become controversial in BMTA-GVHD and PT-GVHD. In syngeneic and autologous bone marrow transplants, GVHD should not occur, according to Billingham's third criterion, but it does. Fischer *et al.* (76) proposed revising the Billingham criteria for the syngeneic cyclosporin-A-induced GVHD mouse model. However, since this has never been proven as GVHD, this proposal is probably incorrect. More recently, Bos *et al.* (71) proposed changing the term BMTA-GVHD to BMTA immune deficiency. This may be more accurate in transfusion-associated GVHD, since transfusions alone are immunosuppressive and proceeds to *in vitro* and *in vivo* diminished recipient immune function in otherwise normal individuals (158, 159).

The incapability of the recipient in rejecting the donor cells is currently under controversy in transfusion-associated GVHD. With the recent cases of PT-GVHD occurring in supposedly immune competent individuals (2–9), the third Billingham criterion appears unnecessary for the pathogenesis of GVHD. Instead, this has been explained and resolved by homozygous HLA haplotypes, because the recipients were unable to recognize and resist the donor lymphocytes. This human example is thought to be identical to the P-into-F_1 hybrid mouse model. In the P-into-F_1 studies, the F_1 host is theoretically tolerant of the donor antigens and cannot reject parent donor cells, allowing the donor cells to attack the alloantigens in the F_1 host cells. Vogelsang (160) described the offspring in this situation as being immunologically normal, and reported that immunologic competence of the host is irrelevant. However, in many of these P-into-F_1 models, the F_1 animals are immunoincompetent through the use of irradiation (Tables I and II), or are neonatal mice with immature immune systems. When apparently older, healthy F_1 mice are used, large doses ($>10^8$) of immunocompetent cells must be inoculated into the host to develop acute-lethal GVHD. When less than 6×10^7 immunocompetent cells are used, they do not develop clinical GVHD, but do have evidence of *in vitro* immune suppression (14).

The human cases of homozygous haploidentical PT-GVHD do not completely conform to the P-into-F_1 model by the MHC genetics, since humans are not inbred. It does conform to the P-into-F_1 model via immune incompetence of the host and dose of transfused immunocompetent cells. With the exception of the case reports by Thaler *et al.* (6), Otsuka *et al.* (8) and Ito *et al.* (105), all of the homozygous haploidentical PT-GVHD cases had immunosuppressed recipients reflected in lymphocytopenia, use of immune suppressive therapy, disease state, or high doses of viable lymphocytes (Table IV).

There has been little emphasis on the immunologic status of the surgical recipient. In the case of Capon *et al.* (104), the patient's absolute lymphocyte count was less the 1.0×10^9/liter, the minimum value used in adults defining lymphocytopenia. The immune system was also depressed due to carcinoma. The patient had a depressed immune system incapable of responding to homozygous haploidentical transfused lymphocytes. Ito *et al.* s (105) case may have been immune depressed by the disease process (cancer), but did reflect a normal lymphocyte count. Since further evaluation of the patient's immune system was not studied, we can only presume that the patient was possibly immune depressed by the disease process. The case that was most normal according to the information in the report was the cholecystectomy case reported by Otsuka *et al.* (8). However, the estimated lymphocyte count was low and possibly less than 1.0×10^9/liter. Unless the patient's immune system was fully evaluated in this case, this patient should be considered presumed immune depressed rather than presumed immunocompetent. Although PT-GVHD has occurred in patients undergoing gastrointestinal surgery, most of the PT-GVHD surgical cases occurred in open-heart and vascular surgery. In Japan, these patients were immunocompromised by several methods. The report by Ino *et al.* (2) indicated that open-heart surgery patients in Japan received 1000–2000 mg of hydrocortisone or methylprednisolone prior to surgery as a cell-membrane stabilizer. All patients received fresh red cells and platelets within 3 days of collection for improved oxygenation and hemostasis. Both of these products contain highly viable lymphocytes. All cases received massive blood transfusions of 800–3400 ml perioperatively. This assured very high lymphocyte doses ($>10^{10}$).

Open-heart surgery provokes immune suppression (161). Why this occurs is unclear, but during the first week after surgery, it was observed that these patients have lymphopenia and depressed cell-mediated immunity. The lymphopenia mainly affects helper T cells (162). Depression of cell-mediated immunity after open-heart surgery increases susceptibility to viral infections (163–165). The postperfusion syndrome is associated with cytomegalovirus (165).

The presence of transient cellular immune deficiency in open-heart surgery also explains why one patient developed PT-GVHD after receiving major HLA mismatched blood (3). This patient engrafted lymphocytes which are normally rejected. This situation could only arise from an immune-depressed host, as shown in Table III.

Table IV lists all of the case reports that were HLA typed. It is clear that nearly all patients had some form of depressed immune system, reflected in lymphocytopenia, cytoreduction agents, or underlying disease. At least 50% of the patients had lymphocytopenia, either actual or estimated. Ten of the 14 cases had lymphoma or carcinoma.

Lymphocytopenia can be found in nearly 50% of Hodgkin's disease cases and is more common in advanced disease (166). T cell function is decreased. All stages of disease have diminished *in vitro* function using lymphocyte stimulation tests to mitogens such as PPD, phytohemagglutinin and concanavalin A (167). The allogeneic MLR is normal in Hodgkin's disease. However, when the MLR is performed with autologous cells, the reaction is significantly depressed (168). *In vivo* T cell function is also depressed, but correlates with stage of disease and is generally decreased with advanced stages (169). Homograft rejection is also delayed in Hodgkin's disease patients (170). Although most every *in vivo* and *in vitro* test shows evidence of a depressed immune system in Hodgkin's disease and non-Hodgkin's lymphomas, the T cell enzyme adenosine deaminase levels correlate with congenital immune deficiencies. Decreased levels of adenosine deaminase have been reported in T cells from Hodgkin's disease patients as well as from patients whose primary defect is congenital immunodeficiency (171). Immune depression from the disease, coupled with cytoreduction agents, is a perfect circumstance for the development of transfusion-induced GVHD.

Corticosteroids are widely used in clinical medicine as agents in the treatment of a diversity of diseases, especially allergic, inflammatory, and immunologic diseases, and as chemotherapeutic agents. The immunosuppressive actions corticosteroids have on the host may be germane to permit the development of PT-GVHD. While the molecular basis of anti-inflammatory action is to date at least partially understood, knowledge regarding the mechanism underlying glucocorticoid effects on the immune system is fragmentary. Immunosuppression thus far is attributed to at least three distinct processes. Inhibition of the production of growth mediators, interference in mediator effects, and glucocorticoid-induced cell death (172). The inhibitory effect on B cell function can be observed both as decreased serum levels of immunoglobulins and as impaired binding of antibodies and complement to the cellular surface (173). Early studies on T cell function indicated lymphocytopenia (174, 175). Impaired stimulation of phytohemagglutinin, concanavalin A, and pokeweed mitogen was diminished with high doses of hydrocortisone (400 mg), but not with standard doses (100 mg) (174).

The effects of corticosteroids on the immune response have more recently been understood. The immune response process is initiated by the interaction of an antigen with the antigen-processing cell, e.g., a macrophage. The macrophage ingests and processes antigens that reappear on the cell surface together with the major histocompatibility antigens. A key cytokine secreted by the macrophage is IL-1. Glucocorticoids interfere with macrophages in processing and displaying antigens (176), inhibiting synthesis and release of IL-1 (177), and blocking IFN-γ's action on macrophages (178). By blocking IL-1, T cells are indirectly incapable of becoming activated.

Glucocorticoids also directly affect T cells which release a series of lymphokines. Specifically, steroids suppress amplification of cell-mediated immunity by inhibiting the expression of the IL-2 gene in T cells and by interfering with the interaction of IL-2 with its receptors on T cells (179). In addition to effects secondary to the suppression of IL-2 synthesis, steroids interfere with the activation of cytotoxic T lymphocytes by IL-2 as well as inhibit the function of natural killer lymphocytes (180).

The mechanism of glucocorticoid-induced cell death can be divided into two steps: a reversible growth inhibition and cell lysis (death). The first step is characterized by many metabolic alterations typical of the catabolic potential of steroids. The lytic mechanism is controversial and speculative. Steroids appear to activate endonucleases that fragment DNA (181). In opposition to the chromatin damage, poly(ADP-ribosyl)ation is activated in order to stabilize the chromatin structure until the antagonistic potential is exhausted and the cells die (182). Whether internucleosomal DNA cleavage is due to endogenous endonuclease(s) (183) or induction of a lytic gene via a steroid membrane receptor which induces a new nuclease(s) is not clear (181, 184). Nevertheless, steroids are lytic to lymphocytes and especially thymocytes (185). This paired with immune regulation dysfunction and lymphocytopenia may depress the host's immune system enough to cause PT-GVHD in what appears to be an immunocompetent patient. Therapeutic doses and even mild to moderate pulse doses probably do not cause significant immunosuppression for the occurrence of PT-GVHD. However, very high pulse doses (1500–2000 mg of hydrocortisone) and prolonged high doses will be immunosuppressive to allow the occurrence of PT-GVHD (186).

Cyclophosphamide is a known immunomodulating agent. It is mostly selective for inhibiting B cells and immunoglobulin synthesis (187). Much less is known about high dose therapy and the effect on cell-mediated immunity. Using chicks that have an identifiable bursa and a thymus, 200 mg/kg drug levels showed complete inhibition of B cells in the bursa and T cells in the thymus. At very low doses (10 mg/kg), only the bursa and B cells were affected (187). Single high dose therapy (over 300 mg/kg) severely, but transiently, depresses T cell function, cytotoxic T cells, helper T cells, and NK cells (188). Some of these functions only return to normal after 5 weeks. At least three cases in Table IV received high doses of cyclophosphamide (102, 110, 112). Cyclophosphamide was not the only cytoreduction agent in these cases. All of

the cases received combination chemotherapy and corticosteroid. The effects of intensive intermittent chemotherapy on host defense mechanisms show marked immune suppression (189). All of this cytoreductive therapy often occurs at the time of blood and platelet transfusions, which have significant doses of immunocompetent cells to cause PT-GVHD. These situations of high dose chemotherapy or steroid therapy require prophylactic blood product irradiation for the recipient, unless one can prove that the recipient is unquestionably immunocompetent.

The recipient's age is also an important immunologic factor. Premature neonates have underdeveloped immune systems that predispose them to PT-GVHD (190). Old age also diminishes the immune response (190). Although age is not an apparent factor in any of the cases reported here (Table IV), it may be influential in the surgical cases of PT-GVHD. All of the surgical cases of PT-GVHD occurred in patients over 50 years of age, with over 70% of the patients in their 60s and 70s (2–9, 104, 105). Also, when considering a multifactorial approach for immune suppression in the host as a cause for PT-GVHD, aging immune incompetence is another additive factor.

The absolute number and subsets of lymphocytes in blood products are important in initiating PT-GVHD. Fresh cellular blood products will obviously assure optimal viability of helper and cytotoxic T lymphocytes. Lymphocyte antigenicity and function remain adequate for 2 weeks in stored blood. However, T cells and mitogenic response begin deteriorating after 1 week of storage (191). Viable, functional T cells are essential in the pathogenesis of PT-GVHD; however, a threshold number requirement is apparent from mice models. The threshold is around 1×10^7. In humans, we see similar findings. In immune refractoriness to platelet transfusion, numbers less than 1×10^7 cause very little alloimmunization (192, 193). PT-GVHD should be very similar, but no clinical studies have dared evaluate this because PT-GVHD is highly lethal. Nevertheless, we know $2–5 \times 10^7$ lymphocytes in the mouse cause GVHD. How these numbers transfer from the animal model to humans is unclear, but it is apparent that 1×10^9 lymphocytes readily cause GVHD in humans. All of the cases in Table IV have lymphocyte doses of nearly 1×10^{10} cells or more. This is a large number of cells given an expected threshold of 1×10^7 to cause GVHD. In the evaluation of all the Japanese PT-GVHD cases, we found that over 1×10^{10} lymphocytes were transfused with 3–5-day-old blood. When evaluating all of the PT-GVHD cases, nearly 50% received fresh (less than 24 hr) random or family-related granulocyte transfusions, which contain over 1×10^{10} lymphocytes. Since the use of granulocyte transfusion has diminished in clinical medicine, the incidence of PT-GVHD may likewise decrease.

Conclusions and Future Directions

PT-GVHD has been known for nearly three decades, but it continues to cause death from blood transfusions. Irradiation of blood products began in the early 1980s to prevent PT-GVHD, yet PT-GVHD persists. It continues because we are unable to identify all patients at risk and because of ineffectiveness in promoting irradiated blood products. Most recently, emphasis has been placed on whose donor blood to irradiate (10) rather than what patients require irradiated blood.

We have shown that patients who otherwise appear healthy are immunocompromised, and with minimal immunocompromisation, require less immunogenetic disparity to cause PT-GVHD. More attention to absolute lymphocyte counts and T lymphocyte subsets in the host is useful in determining the immune status of the patient.

It is apparent that patients with conditions listed in Table V should be evaluated for their immune status and appropriately administered irradiated cellular blood products. The first four absolute indications are definite indications, although according to the survey by Anderson *et al.* (116), these patients do not universally receive irradiated products. Irradiating products for premature infants have been controversial; however, the animal models presented here and the increase in reported cases in humans suggest that this should be an absolute indication. Lymphocytic leukemia patients also have an increased incidence for PT-GVHD than

Table V. Indications for Blood Product Irradiation

Absolute indications
1. Allogeneic and autologous bone marrow transplant recipients[a]
2. Congenital immune deficiency syndromes[a]
3. Hodgkin's disease[a]
4. Intrauterine and exchange transfusions[a]
5. Premature infants[a]
6. Acute lymphocytic leukemia[a]
7. Any patient diagnosed with lymphoma or solid tumors who receives high doses of immunosuppressive drugs and/or irradiation[a]
8. All directed donations, especially first-degree-relative-directed donations
9. Maternal platelet transfusions in neonatal alloimmune thrombocytopenia
Relative indications
1. Any patient receiving high doses of Cytoxan and/or long-term or high-dose steroid therapy
2. Acute nonlymphocytic leukemia
3. Massive transfusions
4. Chronic lymphocytic or myelogenous leukemia
5. Aplastic anemia
No indications
1. Acquired immune deficiency syndrome
2. Thalassemia/sickle cell anemia
3. Hemophiliacs
4. Term newborns

[a] For recommended indications, see Ref. 1.

do nonlymphocytic leukemia patients, perhaps due to the cytoreductive agents used in their treatment.

As shown in Table IV, any patient diagnosed with lymphoma or solid tumors who receives high doses of immunosuppressive drugs and/or irradiation should receive irradiated blood products. Since PT-GVHD is a lethal disease and irradiation to blood products does not cause adverse effects, it is safer to irradiate blood products for these patients.

The last two absolute indications are somewhat controversial to immunohematologists. Certainly blood products from first-degree relatives should be irradiated due to the high probability of homozygosity. This is supported here by animal models and human reports; however, the immune competence of the recipient is refuted. In our own institution, we also irradiate all directed donor blood because of name changes through marriage and because of high homozygous haplotype frequencies in certain races (Japanese, Germans, Jews, Amish, etc) (194). By irradiating all directed donor blood products, we eliminate the possibility of PT-GVHD.

Administering a mother's platelets to her neonate with alloimmune thrombocytopenia, an accepted medical practice, could lead to PT-GVHD if the platelets are not irradiated. This is the only human scenario that best fits the murine model of P-into-F_1 hybrid. The mother must be homozygous at all MHC loci, which was partially true with one case report (108), and the neonate must receive high doses of immunocompetent cells or have a congenital or acquired immune deficiency. The fact that these neonates are immunologically healthy and do not receive large doses of immunocompetent cells may explain why only three cases are reported in the world's literature (106–108).

Relative indications require further documentation, since few cases of PT-GVHD resulted in patients with these diseases. It may also be beneficial to evaluate the patient's immune system in these diseases. If there is any evidence of immune dysfunction or deficiency, these patients should receive irradiated blood products.

Those diseases that have no indications for blood product irradiation are listed in Table V. The chronically transfused thalassemia patients have obviously received MHC homozygous unirradiated blood during their long-term red blood cell support, yet none have reported PT-GVHD. These patients further support the evidence presented here that patients who are immunologically competent do not develop PT-GVHD.

All of the patients at risk for PT-GVHD were documented in the early 1980s; however, additional patients at risk were discovered in surgical patients and patients receiving blood from first-degree-relative-directed donors. It is imperative that cellular blood products be irradiated to prevent PT-GVHD. Leukocyte depletion by filtration or by any other method is insufficient in preventing PT-GVHD (195). All cellular blood products require irradiation, even those that are leukocyte depleted. Many questions have been raised and partially answered, and for the first time, immunopathogenic mechanisms have been proposed. Whether this leads to additional patients at risk or to the understanding of PT-GVHD, with continued observations and investigations, only time can answer.

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