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Minors Come of Age: Minor Histocompatibility Antigens and Graft-versus-Host Disease

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ABSTRACT

Minor histocompatibility antigens (miHA) are responsible for the occurrence of graft-versus-host disease in the setting of a major histocompatibility complex matched sibling allogeneic stem cell transplantation. These miHA are peptide fragments that are associated with major histocompatibility complex class I or class II antigens. Elegant experiments have led to the molecular characterization of these antigens. Efforts to prevent graft-versus-host disease could be targeted through this pathway by matching for these miHA or by preventing antigen recognition. Alternatively, these miHA could be exploited as targets for a more potent graft-versus-malignancy effect. This area of miHA promises to continue to be an exciting area of continued research.
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KEY WORDS

Minor histocompatibility antigens • Graft-versus-host disease

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) is the treatment of choice for a variety of malignant and nonmalignant disorders [1]. Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality even when transplants are between siblings who are matched at the major histocompatibility complex (MHC) for HLAs [2-7]. GVHD in its acute or chronic form can significantly affect the treatment outcome and the quality of life of long-term survivors [4-7].

GVHD occurs when transplanted donor-derived T cells recognize and react to histoincompatible antigens expressed on recipient cells. GVHD is the direct result of one of the principal functions of the immune system, ie, the distinction of self from nonself, or possibly is a result of some danger signal. Final consequences of the GVHD process are host tissue injuries to varying degrees of clinical severity [3,7]. The fundamentals of GVHD include the transfer of genetically disparate donor-derived T cells into a host incapable of rejecting them [3,8]. Three factors are required for the occurrence of a graft-versus-host (GVH) reaction, as outlined by Billingham [8] in his historical Harvey lecture in 1966. The first requirement for a GVH reaction is that the graft must contain a sufficient number of immunologically competent cells. The second requirement is that the host should have important transplantation isoantigens that are lacking in the graft. Hence, the host seems foreign to the graft and is capable of stimulating donor cells. The third requirement is that the host immune system must be incapable of mounting an effective immune response against the graft, at least for a sufficient time for the latter to manifest its immunologic competence.

Specific host cells are recognized as foreign by the alloreactive donor-derived T lymphocytes. Clinical manifestations of GVHD depend on the degree of donor-host histocompatibility and graft alloreactivity to major host antigens. The aim of this review is to outline the important biological basis of GVHD, with emphasis on minor histocompatibility antigens (miHA) and how these antigens may be explored to improve outcome in patients.

GRAFT-VERSUS-HOST DISEASE

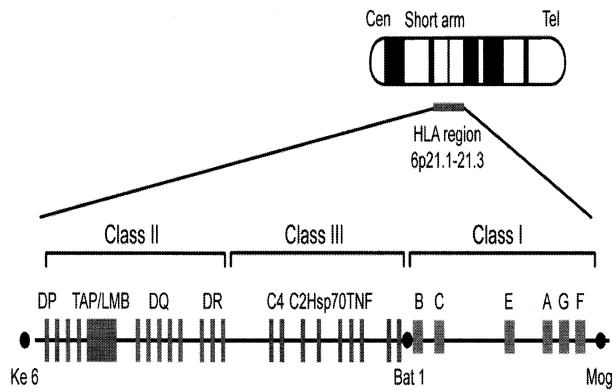GVHD is one of the major risk factors, if not the major risk factor, after allogeneic SCT. In this setting,

Figure 1. Map of the HLA region. The gene map of the HLA region spans approximately 4×10^6 nucleotides and is divided into 3 regions. In general, the class I molecules interact with CD8$^+$ cells and present endogenous antigens, whereas the class II molecules interact with CD4$^+$ cells and present exogenous antigens. Within the class II region also lie the transporter proteins, which are important for antigen presentation. The class III region encodes for the heat shock proteins, tumor necrosis factor, and complement proteins.

it is not difficult to understand that GVHD can occur as immunocompetent cells are transferred into an immunodeficient host. This process of immune recognition is similar in many ways to organ rejection, although in the case of SCT, it is the donor that is rejecting the host. However, in the organ-rejection analogy, the donor and recipient are commonly mismatched at the MHC, and, therefore, the HLAs are different. These antigens are able to trigger T-cell responses, and this leads to graft rejection. Other antigens, such as ABO blood groups and related antigens, can also provoke antibody-mediated rejection, but these are usually excluded from the usual tissue histocompatibility definition because they do not elicit T-cell responses. In the case of an HLA-matched sibling pair, the HLA antigens are genotypically identical, and yet GVHD occurs frequently and can be fatal at times. In this case, T-cell recognition cannot occur through a mismatch at the HLA molecule per se because they are identical.

An explanation for GVHD occurring in the presence of identical HLA molecules came from studies of congenic mice. These are animals that are bred to be identical at the MHC locus but that are disparate in the other areas of their genome. Studies in these animals led to the conclusion that there were other antigens that were important in triggering graft rejection, and these became known as miHAs. It is somewhat of a misnomer to call these antigens minor, because severe GVHD can occur that leads to death. A search for structural antigens similar to those of the MHC was not fruitful, and these antigens remained elusive for several decades. However, it was clear from early murine models that mature donor T cells, of

both CD4$^+$ and CD8$^+$ subsets, could mediate lethal GVHD directed to miHAs [9,10].

GENETIC BASIS OF ACUTE GVHD

Before we discuss the importance of miHA, it is helpful to briefly review the MHC because these HLA molecules underlie the recognition of antigens by T cells. The MHC is highly polymorphic from individual to individual and segregates in families in a Mendelian codominant fashion. Major histocompatibility antigens encoded by the MHC genetic loci have a major effect on transplantation and on the biological progress of GVHD [3,11]. MHC is a closely linked highly polymorphic multigene and multiallelic complex that plays a central role in both cell-mediated and humoral immune responses. MHC genes are found in all mammals and vertebrates and consist of a number of closely linked genetic loci that function as a system (Figure 1). These loci are located on the short arm of chromosome 6 at the p21 position in humans and encodes HLA [12]. Two of the most important distinct classes of cell surface molecules are the class I and II molecules (Figure 2). There are several different types of class I and II molecules. Class I molecules (HLA-A, -B, and -C) are expressed on the surfaces of virtually all nucleated cells at varying densities [3]. HLA class II molecules (HLA-DR, -DQ, and -DP) are expressed primarily on cells of the immune re-
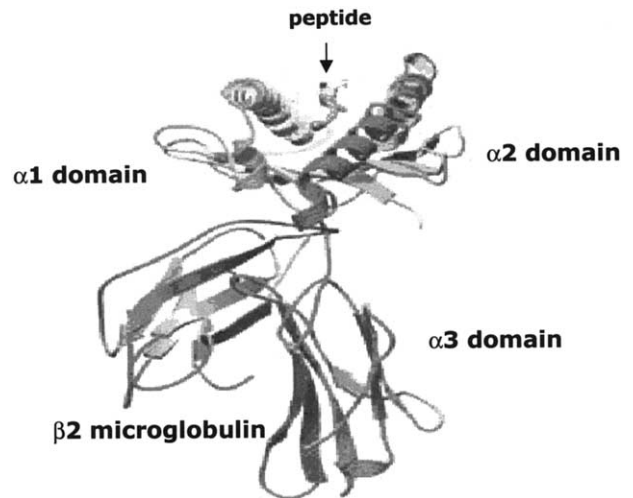

Figure 2. MHC class I molecule. Class I MHC is a membrane-spanning molecule composed of 2 proteins. It is divided into 3 globular domains: α-1, α-2, and α-3; α-1 is closest to the amino terminus, and α-3 is closest to the membrane. The bound peptide sits within the groove created by the 2 α helices. The MHC class II molecule is similarly composed of 2 membrane-spanning proteins and is not associated with β-2 microglobulin. Instead, there are 2 globular domains termed (1) α-1 and α-2 and (2) β-1 and β-2. The 2 regions farthest from the membrane are α-1 and β-1. The 2 chains associate without covalent bonds. The bound peptide likewise sits within the groove.
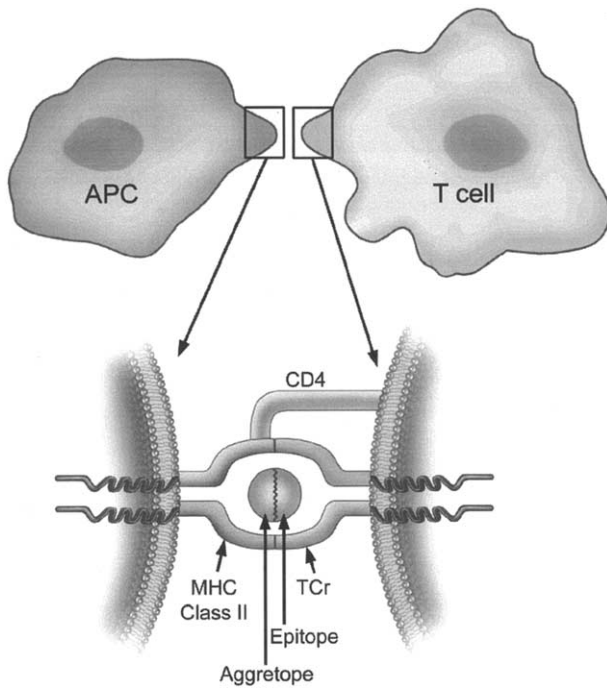
Figure 3. Antigen-presenting cell (APC) and T-cell interaction. The APC presents the peptide antigen (in this case, a minor histocompatibility antigen) to the T-cell receptor. The agretope interacts with the MHC molecule while the epitope interacts with the T-cell receptor.

sponse system, particularly B lymphocytes and antigen-presenting cells (APC) such as monocytes, dendritic cells, and macrophages [3]. However, cytokines secreted by lymphocytes and monocytes during immune activation may cause dramatic increases in class II HLA antigen expression, even on cell types that normally have little or no surface expression. HLA-A, -B, and -DR molecules seem to be the most important loci for determining whether transplanted cells initiate a GVH reaction [3,12-14]. Matching SCT recipients with sibling donors sharing identical HLA molecules significantly improved engraftment kinetics and decreased GVHD severity [1,15,16]. $CD4^+$ and $CD8^+$ T cells recognize foreign antigens via their presentation by class II and class I HLA molecules, respectively (Figure 3). The structure of the MHC class II molecule is similar to that of the class I molecule [17].

Although there is no doubt that HLA matching is important and that miHAs are also important, GVHD is not simply the result of mismatching. Although it is likely that a GVH reaction always occurs in the case of mismatching, whether the disease itself occurs is related to many other factors, such as the preparatory regimen (the type and dose of radiation, chemotherapy, or both), the amount of prior therapy, past cytomegalovirus exposure, and the GVHD prophylaxis regimen [7]. Moreover, the contribution of selected cytokines to this disease process has been elucidated

recently [5,18]. Other factors, such as the ability to present the specific antigen or the ability of an activated T cell to traffic and home to a particular tissue, may also play an important role in whether GVHD ultimately occurs [19]. Although miHA is critical to the induction of GVHD, other factors contribute to ultimately determine whether the disease occurs. This review will primarily discuss the initial antigen recognition and downstream events. However, it is important to remember that there are cellular and cytokine factors that are in a balance between the induction of GVHD from the activated T cells and the control of GVHD through regulatory T cells [20,21].

ANTIGEN PRESENTATION

HLA molecules provide the crucial surface upon which T-cell receptors (TCRs) recognize foreign (nonself) antigens. These antigens come from proteins that are broken down in to peptide fragments and presented by class I or II molecules on the surface of the APC. Proteins routinely undergo processing into smaller peptide fragments within the cell; the mechanism differs with the actual location of the proteins. In the case of HLA class I–restricted antigens, the intracellular protease machinery (proteasome) must first degrade cytoplasmic proteins into fragments, usually of 8 to 11 amino acids. These peptides are then transported to the endosome compartment by transporter molecules (transporter in antigen processing). In the endoplasmic reticulum, the peptides bind to available class I molecules, and the assembled complexes are transported to the cell surface [22].

Class II molecules function primarily to present peptides stemming from the external milieu of the cell. Class II molecules present antigenic fragments (in the form of linear peptides) to the $CD4^+$ inducer (or helper) T cells, whereas class I molecules function at the effector phase of immunity by presenting antigens to $CD8^+$ T cells, which generally have cytotoxic function. This process of antigen presentation consists of the binding of a single TCR to a complex on the surface of an APC that consists of the MHC molecule and a peptide fragment derived from the foreign antigen. In an allogeneic SCT, the principal antigenic targets of the T cells of the graft are the host HLA molecules if the patient and donor HLA molecules differ. However, for grafts matched at the HLA, the different peptides sitting in the binding groove of the HLA molecule, termed miHA, seem to underlie the development of GVHD [3].

Minor differences in these peptide sequences as compared with the native peptide (such as substitution of 1 amino acid) can also have profound consequences on the outcome of TCR/peptide/MHC interactions, possibly resulting in partial activation [22,23]. TCR

interactions therefore do not simply activate or disable a T cell; rather, in response to subtle variations of peptide sequences, this engagement may cause a multitude of responses that range from profoundly helpful to deleterious. These peptide differences may cause changes in the binding of the TCR or alter its binding to the HLA molecule. Moreover, peptide differences may have a significant effect on how the peptide is initially processed through the proteosome complex. Thus, these differences in processing may account for antigen presentation being possible in the recipient but not in the donor. In such a case, donor T cells may not be educated to such a particular antigen as "self" and, therefore, can mount an immune response against such an antigen present on the recipient cells. Several miHAs seem to function in this manner [24-26]. Small changes in peptides may shift a vigorous proliferative response to the induction of an anergic state (or vice versa). These changes have been referred to as altered peptide ligands [27].

MINOR HISTOCOMPATIBILITY ANTIGENS

When the SCT donor and the recipient are MHC identical, alloreactivity can occur through the TCR recognition by donor T cells of different host-derived peptides bound to the self-same MHC molecules on host APCs. These are the so-called miHAs and are likely derived from a wide range of endogenous proteins (eg, transferrin and myoglobin) that may possess genetic polymorphisms between individuals [28]. Because T cells do not recognize antigens alone, but in conjunction with the MHC (usually self) on an APC [29], miHAs are bound and presented in the clefts of the MHC molecules, and these complexes are then recognized by TCRs of individual specific donor T cells. Alloreactivity is thus the combined effect of recognition by numerous donor T cells of different nonself host peptides presented by MHC molecules on APCs [30,31]. Presentation of miHA (host peptide) by the MHC class II molecules leads to activation of donor CD4+ T cells, and, likewise, presentation by class I molecules induces responses of CD8+ T cells. GVHD, and the immunopathology associated with it, develops as a consequence of these donor T-cell responses to the host miHA.

The other variable in the induction and development of GVHD is the target or tissue distribution of the miHA. Some are distributed broadly throughout somatic tissues, whereas others seem to be lineage restricted. These differences may correlate with the intensity and localization of the immunopathology of GVHD in a given transplantation setting.

Murine Studies

Previous experimental data have demonstrated that miHAs are peptides, and one could use this knowledge to attempt to prevent GVHD. Unrelated peptides (ie, not native to the host and nonimmunogenic) with a strong binding affinity to MHC class II molecules could successfully inhibit a secondary mixed lymphocyte reaction in vitro and prevent GVHD in vivo. The administration of specific peptides with high-affinity binding for their respective MHC class II molecules was capable of preventing GVHD in 2 separate murine models [32]. In both murine models, the mechanism of prevention was found to be MHC associated, because nonbinding peptides had no clinical effects. However, this approach has been limited by the need for allele specificity of the inhibitor peptides and by the difficulty of achieving sustained tissue levels of such low-molecular-weight peptides over a prolonged period after injection.

One possibility for overcoming the concerns expressed previously was to use a larger synthetic polypeptide with promiscuous binding to MHC class II molecules [33,34]. The molecule chosen is a random synthetic amino acid polymer composed of glycine, alanine, lysine, and tyrosine, which we have termed GLAT (also known commercially as Copaxone; Teva Pharmaceutical Industries, Ltd., Jerusalem, Israel); it had been demonstrated to be effective in the prevention and treatment of experimental allergic encephalomyelitis. We next tested this polymer for its utility in the prevention of acute GVHD. GLAT was effective in the prevention of proliferative responses in vitro and was able to prevent GVHD in vivo in a murine model of bone marrow transplantation across minor antigenic differences [35]. These murine studies led to a phase I clinical trial that used this molecule in the therapy of steroid-refractory GVHD (data are presented below).

Characterization of Minor Antigens

The miHAs have been identified by cloning T cells from recipients of allogeneic SCT who developed GVHD. These cloned T cells allow for the characterization of HLA class I–restricted miHAs. The peptides have been primarily characterized through elution from purified class I molecules, which are then fractionated by high-performance liquid chromatography and analyzed by mass spectroscopy [24-26,36]. The miHAs termed HA-1, HA-2, and HA-8 and male-specific miHA have been characterized in this manner. Another elegant method to characterize the miHA termed HB-1 was through complementary DNA (cDNA) expression cloning. In these experiments, messenger RNA was isolated from miHA-positive Epstein-Barr virus transformed lymphocytes, and a cDNA library was constructed [37,38]. These cDNAs were then co-transfected with the cDNA of the restricting HLA molecule and transfected into COS-1 cells. The readout for the expres-

sion of the antigen was through the ability to stimulate a specific cytotoxic T lymphocyte (CTL) to produce tumor necrosis factor-α.

The search for class II binding miHAs has been more difficult because the processing involves several other molecules, such as the invariant chain, which is important for the stability of the HLA molecule and the transport of the peptide, and HLA-DM, which is important for proper peptide processing. Recently, the first class II–restricted miHA was identified [39,40]. In human studies, the Y-specific genes were placed into a retrovirus and transduced into female HLA-DQ5$^+$ Epstein-Barr virus transformed lymphocytes. A CTL specific for the HLA-DQ5 HY was then used to lyse the transfected cells. Lysis occurred from fragments of the DBY protein, which was used to localize the miHA. Another method that has been successful in the search for class II miHAs is to screen a recombinant bacterial library or COS cells retrovirally transduced with HLA-DM and Ii. In this case, the CTL-specific cell line is again used to screen those clones [41]. However, responses to miHA are likely to be heterogeneous and will vary depending on the genetic background of the individual [42-44].

It is possible that a unique minor antigen, such as one directed against hematopoietic cells, would not lead to GVHD because the expression of restricted antigens to the hematopoietic stem cells should not cause GVHD of the skin or liver. Although it is clear that reactivity to a restricted miHA must be limited to the tissue where the MHC class I are expressed, recent studies in a murine model for MHC disparities demonstrated that the toxicity may be broader than the limited antigen-expressing target [18]. In this murine study, T-cell responses directed specifically at recipient dendritic cell miHAs were able to induce acute GVHD in the absence of broad T-cell responses directed against the target tissues of GVHD, such as the liver. The local inflammatory response results in an excess of cytokine production that is sufficient to cause damage to the organ. However, in models in which GVHD was directed only against miHA, expression by nonhematopoietic tissues seemed required and may be related to the ability of APCs to sustain T-cell stimulation and to the importance of direct target cell cytolysis for tissue damage [45,46].

Clinical Data

Because the manner in which a particular protein is processed is dependent on genes outside of the MHC, 2 siblings, despite having identical MHC molecules, will have different peptides in the MHC groove. The identification of the particular peptides responsible for GVHD has been an area of intense research. The earliest and most logical minor antigen was derived from studies of sex-disparate SCT. In this

case, the male-specific HY antigens have been shown to play an important role. These male-specific antigens have been characterized recently [39,47-50]. It is interesting that these antigens seem to play an important role in the ability of a donor to reject the graft, but there are only limited data to suggest that these antigens play a role in GVHD, perhaps because of where these antigens are expressed. This is also true in murine models, in which many immunodominant miHAs, defined by CTL activity or by skin graft rejection, do not seem to correlate with GVHD potential [43,51,52].

Many potential miHA antigens exist in humans, but the actual number that may cause GVHD is probably limited. One such antigen, the HA-2 peptide, has been found to be a member of the class I myosin family [53,54]. In one clinical study, 5 miHA antigens (HA-1, -2, -3, -4, and -5) recognized by T cells in association with HLA-A1 and -A2 were studied in recipients of bone marrow transplants [55]. Mismatching of HA-1 alone was significantly correlated with acute GVHD (greater than grade II; $P = .02$), and mismatching at HA-1, -2, -4, and -5 was also associated with GVHD ($P = .006$). Acute GVHD developed in all cases in which an HA-1–positive patient received an HA-1–negative graft. HA-1 is also efficient in inducing antigen-specific CTLs [56]. Another possible miHA is the polymorphic residues of CD31. An initial study demonstrated that allelic differences in this molecule were associated with the development of GVHD [57]. These findings have remained somewhat controversial because several, but not all, have confirmed that allelic differences are important in the etiology of GVHD [58,59].

EXPLOITING miHA DIFFERENCES

As mentioned previously, miHAs have been associated with known graft rejection, especially in the case of HY antigen. Cloning T cells responsible for graft rejection led to the discovery of male-specific and other miHAs [50,60,61]. One possible method to exploit these miHAs is to use the miHAs of the malignant cells as targets for T-cell responses. Thus, in the setting of HLA-matched SCT, the responses against miHA of the leukemia cells could be boosted as a method to improve the graft-versus-leukemia (GVL) effect. Laboratory studies have demonstrated that these male-specific antigens, as well as HA-1 through HA-5, HA-8, and HB-1, can be recognized on leukemic precursors by the specific T-cell clones [25,62,63]. These approaches of optimizing GVL responses may be even more important in the setting of nonablative SCT, where the antileukemic effect of the donor cells is the critical component of the procedure. In these cases, the use of donor lymphocyte infusions,

if directed against the specific miHA, could result in important tumor control without excessive toxicity. For example, HA-1 and HA-2 were known to contribute to the occurrence of GVHD, and T cells specific against these antigens were known to kill HA-1– and HA-2–positive leukemic cells [62]. In a series of elegant experiments, donors and recipients who were mismatched at these 2 miHAs were studied for the appearance of T cells that were specific for the miHA by using tetramers of the HLA/peptide complexes. In these studies, the disappearance of the malignant cells coincided with a dramatic increase in the HA-1– and HA-2–specific T cells [64]. In another case, the emergence of HY-specific T cells was also associated with the development of acute GVHD. This recognition process can be found in CD4$^+$ cells as well. In one case of graft rejection in a female patient with aplastic anemia who received a stem cell graft from her brother, HY-specific MHC class II–restricted CD4$^+$ T cells were isolated from the recipient [65]. These T-helper cells mature dendritic cells and can enhance the expansion of MHC class I–restricted CD8$^+$ T cells. This mechanism therefore completes the immune response cycle, with interactions between CD4$^+$ and CD8$^+$ cells and dendritic cells leading to a miHA-specific response.

The use of tetramers to detect these cells also allowed for their isolation. Once isolated, these cells can be expanded in vitro and tested for their ability to lyse specifically leukemic targets, as has been done for GVHD patients [66]. These T-cell clones could then be used for donor leucocyte infusion, resulting in specific tumor responses. Although there could still be a risk of GVHD because of the cytokine production resulting from activation of these cells, the overall incidence should be lower given the more restricted CTL targets. Thus, adoptive transfer of tumor-specific T cells could overcome the delay in the host of mounting a specific, effective immune response, especially in those tumors in which the growth rate is rapid. Moreover, these miHAs could be loaded onto dendritic cells, and the recipient could be specifically immunized against these miHAs as a method to enhance the GVL effect. Another method to generate miHA-specific, leukemia-reactive CTLs has been to use chronic myeloid leukemia cells as stimulators in the presence of α-interferon [67].

Not all targets of GVL are miHAs. Other tissue-specific antigens can also be useful targets for the GVL effect. For example, proteinase-3 and other proteins that are specific in the malignant myeloid cells are clear targets for CTLs [68]. PR-1 peptide, which is derived from proteinase-3, is the target for these T cells. Patients who achieve a complete remission have the concurrent appearance of these cells specific for PR-1 in their circulation. Moreover, inoculation with proteinase-3 peptides resulted in detectable T cells specific for this antigen and in clinical remissions [69].

CLINICAL TRIAL USING ANTIGEN-PRESENTATION BLOCKADE

As previously mentioned, murine studies with GLAT resulted in the binding of this random polymer to the MHC molecule and prevented the recognition of T cells of the miHA differences between 2 congenic strains of mice. In an effort to exploit these finding in the mouse, we have tested this molecule for its utility in humans. We have conducted a clinical phase I trial by using this synthetic polymer, GLAT, for the treatment of steroid-refractory acute GVHD. Twelve patients received GLAT as therapy for acute GVHD at Stanford or Duke University. Five patients had 1 prior acute GVHD therapy, and 7 had 2 to 5 prior acute GVHD therapies. All patients had experienced treatment failure with 4 or more days of corticosteroid treatment at 2 mg/kg/d (9 patients) or 1 mg/kg/d (3 patients) with a calcineurin inhibitor. Five patients had grade III skin-only GVHD. All 5 patients responded: 3 with complete resolution of GVHD (although 1 received concomitant photopheresis), 1 with a sustained partial remission, and 1 with a transient 3-week partial remission. Seven patients had overall grade IV GVHD that involved the skin, gut, or liver. Five of these 7 patients had responses: 3 with complete resolution and 2 with transient resolution of 30 and 50 days. Two patients had no response. Eleven patients died. Four patients died of progressive GVHD with or without infectious complications (2 cytomegalovirus and 1 aspergillus). One patient with complete resolution died with probable pulmonary chronic GVHD. The remaining patients died of relapsed malignancy (n = 3), bacterial infection (n = 2), or interstitial pneumonia (n = 1). One patient was alive 10 months after transplantation without active GVHD. Dose escalation of GLAT from 20 to 80 mg/d was achieved without dose-limiting toxicities. In summary, GLAT has efficacy in patients with steroid-refractory GVHD of the skin, gut, and liver, with a sustained response in 7 of 12 patients and without identifiable toxicity. Unfortunately, the prognosis of patients with steroid-refractory acute GVHD is guarded. In this small phase I trial, GLAT did not improve the expected poor outcome of this patient population. However, these preliminary data may support the use of GLAT earlier in the course of prevention of acute GVHD at a time where blocking antigen presentation through miHA may be more effective.

CONCLUSION

The occurrence of GVHD remains a major barrier to effective SCT and limits its use in many dis-

eases. The antigens responsible for acute GVHD in an HLA-matched SCT are miHAs. These miHAs are peptides from endogenous proteins that are expressed on the host APCs. These peptide differences between the host and the donor lead to donor CTLs reacting against the host antigens, which leads to the resulting damage to the target tissues. One potential for preventing GVHD would be to interfere with the miHA presentation, thereby abrogating the initial step toward T-cell activation. However, these miHAs can be exploited when their expression is restricted, for example, to the malignant leukemic cells. The ability to generate miHA-specific T-cell clones and to isolate and expand these clones against the malignant cells could result in a more effective donor leucocyte infusion. Moreover, vaccine strategies against these miHAs could also improve control of the malignant disease. The technology to identify, isolate, and expand such cells is now available. Clinical trials using this approach of adoptive T-cell therapy or target immunization to such antigens are in their initial phases but promise to be an exciting area of further research.

ACKNOWLEDGMENTS

REFERENCES

1. Thomas ED, Blume KG. Historical markers in the development of allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 1999;5:341-346.

2. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs.-host disease. *Biol Blood Marrow Transplant.* 1999;5:347-356.

3. Chao NJ. *Graft-versus-Host Disease.* Second Edition. Austin, TX: R.G. Landes Co.; 1999.

4. Chao NJ, Schmidt GM, Niland JC, et al. Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease. *N Engl J Med.* 1993;329:1225-1230.

5. Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med.* 1991;324:667-674.

6. Socie G, Stone JV, Wingard J, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. *N Engl J Med.* 1991;341:14-19.

7. Sullivan KM. Graft versus host disease. In: Thomas ED, Blume KB, Forman SJ, eds. *Hematopoietic Cell Transplantation.* Second Edition. Oxford, UK: Blackwell Scientific Publications; 1991, 515-536.

8. Billingham RE. The biology of graft-versus-host reactions. *Harvey Lect.* 1966;62:21-78.

9. Korngold R, Sprent J. Lethal graft-versus-host disease following bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from marrow. *J Exp Med.* 1978;148:1687-1698.

10. Korngold R, Sprent J. Variable capacity of L3T4+ cells to cause lethal graft-versus-host disease across minor histocompatibility barriers. *J Exp Med.* 1987;165:1552-1564.

11. Martin PJ. Overview of marrow transplantation immunology. In: Thomas ED, Blume KB, Forman SJ, eds. *Hematopoietic Cell Transplantation.* Second Edition. Oxford, UK: Blackwell Scientific Publications; 1999, 19-26.

12. Hansen JA, Choo SY, Geraghty DE, Mickelson E. The HLA system in clinical marrow transplantation. *Hematol Oncol Clin North Am.* 1990;4:507-523.

13. Mickelson E, Petersdorf EV. Histocompatibility. In: Thomas ED, Blume KB, Forman SJ, eds. *Hematopoietic Cell Transplantation.* Second Edition. Oxford, UK: Blackwell Scientific Publications; 1999, 28-37.

14. Bach FH, van Rood JJ. The major histocompatibility complex: genetics and biology. *N Engl J Med.* 1976;295:872-877.

15. Martin PJ, Gooley T, Anasetti C, Petersdorf EW, Hansen JA. HLAs and risk of acute graft-vs.-host disease after marrow transplantation from an HLA-identical sibling. *Biol Blood Marrow Transplant.* 1998;4:128-134.

16. Pei J, Martin PJ, Longton G, et al. Evaluation of pretransplant donor anti-recipient cytotoxic and helper T lymphocyte responses as correlates of acute graft-vs.-host disease and survival after unrelated marrow transplantation. *Biol Blood Marrow Transplant.* 1997;3:142-149.

17. Brown JH, Jardetzky TS, Gorga JC, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature.* 1993;364:33-37.

18. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med.* 2002;8:575-581.

19. Fuhlbrigge RC, Kieffer JD, Armerding D, Kupper TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-I expressed on skin-homing T cells. *Nature.* 1997;389:978-981.

20. Levings MK, Sangregorio R, Sartirana C, et al. Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J Exp Med.* 2002;196:1335-1346.

21. Taylor PA, Lees CJ, Blazar BR. The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood.* 2002;99:3493-3499.

22. Van den Eynde BJ, Morel S. Differential processing of class-I-restricted epitopes by the standard proteasome and the immunoproteasome. *Curr Opin Immunol.* 2001;13:147-156.

23. Pober JS, Gimbrone MA Jr, Lapierre LA, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J Immunol.* 1986;137:1893-1899.

24. den Haan JM, Sherman NE, Blokland E, et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science.* 1995;268:1476-1479.

25. Pierce RA, Field ED, Mutis T, et al. The HA-2 minor histocompatibility antigen is derived from a diallelic gene encoding a novel human class I myosin protein. *J Immunol.* 2001;167:3223-3231.

26. Brickner AG, Warren EH, Caldwell JA, et al. The immunogenicity of a new human minor histocompatibility antigen results

from differential antigen processing. *J Exp Med.* 2001;193:195-206.

27. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu Rev Immunol.* 1996;14:1-27.

28. Claverie JM, Kourilsky P, Langlade-Demoyen P, et al. T-immunogenic peptides are constituted of rare sequence patterns. Use in the identification of T epitopes in the human immunodeficiency virus gag protein. *Eur J Immunol.* 1988;18:1547-1554.

29. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of graft-vs.-host disease by inactivation of host antigen presenting cells. *Science.* 1999;285:412-415.

30. Hopkins KA, Vogelsang GB, Delaney NL, Gullette DL, Santos GW, Bias WB. Implication of a gene distal to HLA-A in the etiology of graft-versus-host disease. *Transplant Proc.* 1989;21:2971-2972.

31. Chao NJ. Graft-versus-host disease: the viewpoint from the donor T cell. *Biol Blood Marrow Transplant.* 1997;3:1-6.

32. Schlegel PG, Aharoni R, Smilek DE, et al. Prevention of graft-versus-host disease by synthetic peptides with high binding affinity for class II MHC molecules. *Blood.* 1994;84:2802-2810.

33. Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol.* 1971;1:242-248.

34. Teitelbaum D, Webb C, Meshorer A, Arnon R, Sela M. Suppression by several synthetic polypeptides of experimental allergic encephalomyelitis induced in guinea pigs and rabbits with bovine and human basic encephalitogen. *Eur J Immunol.* 1973;3:273-279.

35. Schlegel PG, Aharoni R, Chen Y, et al. Asynthetic random basic copolymer with promiscuous binding to class II major histocompatibility complex molecules inhibits T-cell proliferative responses to major and minor histocompatibility antigens in vitro and confers the capacity to prevent murine graft-versus-host disease in vivo. *Proc Natl Acad Sci U S A.* 1996;93:5061-5066.

36. den Haan JM, Meadows LM, Wang W, et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science.* 1998;279:1054-1057.

37. Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P, Van Pel A. Tumor antigens recognized by T lymphocytes. *Annu Rev Immunol.* 1994;12:337-365.

38. Dolstra H, Fredix H, Maas F, et al. A human minor histocompatibility antigen specific for B cell acute lymphoblastic leukemia. *J Exp Med.* 1999;189:301-311.

39. Vogt MHJ, van den Muijsenberg JW, Goulmy E, et al. The DBY gene codes for an HLA-DQ5-restricted human male-specific minor histocompatibility antigen involved in graft-versus-host disease. *Blood.* 2002;99:3027-3035.

40. Scott D, Addey C, Ellis P, et al. Dendritic cells permit identification of genes encoding MHC class II-restricted epitopes of transplantation antigens. *Immunity.* 2000;12:711-719.

41. Sahara H, Shastri N. Second class minors: molecular identification of the autosomal H46 histocompatibility locus as a peptide presented by major histocompatibility complex class II molecules. *J Exp Med.* 2003;197:375-384.

42. Friedman TM, Statton D, Jones SC, Berger MA, Murphy GF, Korngold R. Vbeta spectratype analysis reveals heterogeneity of CD4+ T-cell responses to minor histocompatibility antigens involved in graft-versus-host disease: correlations with epithelial tissue infiltrate. *Biol Blood Marrow Transplant.* 2001;7:2-13.

43. Korngold R, Leighton C, Mobraaten LE, Berger MA. Inter-strain graft-vs.-host disease T-cell responses to immunodominant minor histocompatibility antigens. *Biol Blood Marrow Transplant.* 1997;3:57-64.

44. Friedman TM, Gilbert M, Briggs C, Korngold R. Repertoire analysis of CD8+ T cell responses to minor histocompatibility antigens involved in graft-versus-host disease. *J Immunol.* 1998;161:41-48.

45. Korngold R, Sprent J. Features of T cells causing H-2 restricted lethal graft-vs-host disease across minor histocompatibility barriers. *J Exp Med.* 1982;155:872-883.

46. Jones SC, Friedman TM, Murphy GF, Korngold R. Importance of minor histocompatibility antigen expression by non-hematopoietic tissues in a CD4+ T-cell-mediated graft-versus-host disease model. *J Clin Invest.* 2003;112:1880-1886.

47. Vogt MH, Goulmy E, Kloosterboer FM, et al. UTY gene codes for an HLA-B60-restricted human male-specific minor histocompatibility antigen involved in stem cell graft rejection: characterization of the critical polymorphic amino acid residues for T-cell recognition. *Blood.* 2000;96:3126-3134.

48. Wang W, Meadows LR, den Haan JM, et al. Human H-Y: a male-specific histocompatibility antigen derived from the SMCY protein. *Science.* 1995;269:1588-1591.

49. Warren EH, Gavin MA, Simpson E, et al. The human UTY gene encodes a novel HLA-B8- restricted H-Y antigen. *J Immunol.* 2000;164:2807-2814.

50. Goulmy E, Termijtelen A, Bradley BA, van Rood JJ. Y-antigen killing by T cells of women is restricted by HLA. *Nature.* 1977;266:544-546.

51. Korngold R, Wettstein PJ. Immunodominance in the graft-versus-host disease T cell response to minor histocompatibility antigens. *J Immunol.* 1990;145:4079-4088.

52. Blazar BR, Roopenian DC, Taylor PA, Christianson GJ, Panoskaltsis-Mortari A, Vallera DA. Lack of GVHD across classical, single minor histocompatibility (miH) locus barriers in mice. *Transplantation.* 1996;61:619-624.

53. den Haan JM, Sherman NE, Blokland E, et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science.* 1995;268:1476-1479.

54. Pierce RA, Field ED, Mutis T, et al. The HA-2 minor histocompatibility antigen is derived from a diallelic gene encoding a novel human class I myosin protein. *J Immunol.* 2001;167:3223-3229.

55. Goulmy E, Schipper R, Pool J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med.* 1996;334:281-288.

56. Mutis T, Ghoreschi K, Schrama E, et al. Efficient induction of minor histocompatibility antigen HA-1-specific cytotoxic T-cells using dendritic cells retrovirally transduced with HA-1-coding cDNA. *Biol Blood Marrow Transplant.* 2002;8:412-419.

57. Behar E, Chao NJ, Hiraki DD, et al. Polymorphism of adhesion molecule CD31 and its role in acute graft-versus-host disease. *N Engl J Med.* 1996;334:286-292.

58. Grumet FC, Hiraki DD, Brown BWM, et al. CD31 mismatching affects marrow transplantation outcome. *Biol Blood Marrow Transplant.* 2001;7:503-512.

59. Balduini CL, Frassoni F, Noris P, et al. Donor-recipient incompatibility at CD31-codon 563 is a major risk factor for acute graft-versus-host disease after allogeneic bone marrow

transplantation from a human leucocyte antigen-matched donor. *Br J Haematol.* 2001;114:951-953.

60. Nichols WC, Antin JH, Lunetta KL, et al. Polymorphism of adhesion molecule CD31 is not a significant risk factor for graft-versus-host disease. *Blood.* 1996;88:4429-4434.
61. Marijt WA, Kernan NA, Diaz-Barrientos T, et al. Multiple minor histocompatibility antigen-specific cytotoxic T lymphocyte clones can be generated during graft rejection after HLA-identical bone marrow transplantation. *Bone Marrow Transplant.* 1995;16:125-132.
62. Falkenburg JHF, Goselink HM, van del Harst D, et al. Growth inhibition of clonogenic leukemic precursor cells by minor histocompatibility antigen-specific cytotoxic T lymphocytes. *J Exp Med.* 1991;174:27-35.
63. Dolstra H, Fredrix H, Preijers F, et al. Recognition of a B cell leukemia-associated minor histocompatibility antigen by CTL. *J Immunol.* 1997;158:560-569.
64. Marijt WA, Heemskerk MH, Kloosterboer FM, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-l- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci U S A.* 2003;100:2742-2748.
65. Spierings E, Vermeulen CJ, Vogt MH, et al. Identification of HLA class II-restricted H-Y-specific T-helper epitope evoking CD4+ T-helper cells in H-Y-mismatched transplantation. *Lancet.* 2003;362:610-615.
66. Mutis T, Gillespie G, Schrama E, Falkenburg JH, Moss P, Goulmy E. Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. *Nat Med.* 1999;5:839-846.
67. Faber LM, van Luxemburg-Heijs SA, Rijnbeek M, Willemze R, Falkenburg JH. Minor histocompatibility antigen-specific, leukemia-reactive cytotoxic T cell clones can be generated in vitro without in vivo priming using chronic myeloid leukemia cells as stimulators in the presence of alpha-interferon. *Biol Blood Marrow Transplant.* 1996;2:31-36.
68. Molldrem JJ, Lee PP, Wang C, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med.* 2000;6:1018-1024.
69. Molldrem JJ, Komanduri K, Wieder E. Overexpressed differentiation antigens as targets of graft-versus-leukemia reactions. *Curr Opin Hematol.* 2002;9:503-508.