# Overexpressed differentiation antigens as targets of graft-versus-leukemia reactions

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The graft-versus-leukemia (GVL) effect associated with allogeneic blood and marrow transplantation has largely been a clinically described phenomenon until recently. We are beginning to understand the cellular and molecular nature of GVL, and in this review the authors highlight the potential for self-antigen–specific T lymphocytes to contribute to GVL. The authors focus on myeloid tissue–restricted proteins as GVL target antigens in CML and AML, and in particular on proteinase 3 and other azurophil granule proteins as targets for both autologous and allogeneic T-cell responses. Finally, the authors discuss myeloid self-antigen–directed alloreactivity in the context of our evolving understanding of the critical molecular determinants of allogeneic T-cell recognition. By altering T-cell receptor affinity, peptide specificity can be maintained and the potency of immunity can be enhanced in the MHC-mismatched setting. Curr Opin Hematol 2002, 9:503–508



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**Abbreviations**

| | |
|---|---|
| **CFU-GM** | colony-forming unit granulocyte-macrophage |
| **CML** | chronic myelogenous leukemia |
| **CTL** | cytotoxic T lymphocytes |
| **DLI** | donor lymphocyte infusions |
| **GVHD** | graft-versus-host-disease |
| **GVL** | graft-versus-leukemia |
| **mHA** | minor histocompatibility antigen |
| **MPO** | myeloperoxidase |
| **PR1-CTL** | CTL specific for PR1 |
| **Pr3** | proteinase 3 |
| **TCR** | T-cell receptor |

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The power of allogeneic lymphocytes to cure malignancies is perhaps best demonstrated by what happens to patients who receive donor lymphocyte infusions (DLI) for relapsed chronic myelogenous leukemia (CML). As many as 80% of these patients achieve a molecular remission [1,2], an effect that has been termed graft-versus-leukemia, or GVL. This is mediated mostly by T lymphocytes, because depletion of T cells from the graft abrogates this effect. Unfortunately, another T-cell–mediated effect, graft-versus-host-disease (GVHD), accompanies DLI therapy in up to 50% of patients, thus limiting the full therapeutic potential of DLI. Because many patients achieve remission during flares of GVHD, it is uncertain whether GVL can be separated from GVHD or whether these phenomena are irrevocably linked. However, up to 55% of patients that do not develop GVHD also achieve molecular remission, suggesting that these immune reactions are separable [1,3]. If there were distinct effector cells or unique target antigens for the effector cells that produced GVL versus GVHD, then the full therapeutic potential of allogeneic DLI might be realized by treatment strategies that took advantage of these differences.

## Identifying differentiation antigens as GVL targets

The range of target antigens for allogeneic donor lymphocytes includes HLA molecules, minor histocompatibility antigens (mHAs), or self-antigens that are aberrantly expressed in the tumor compared with normal tissues. In the case of HLA-matched BMT, alloreactivity directed against polymorphic mHA could account for both GVL and GVHD. Under these circumstances, the tissue distribution of the target mHA would direct the type of immune reaction. Certain mHA that have expression restricted to the tissue from which the tumor is derived but not other host tissues might therefore also be ideal target antigens for preferential T-cell reactivity (Fig. 1) against the tumor leading to graft-versus malignancy. This would require binding of the mHA to the HLA molecule with threshold recognition by T cells that have T-cell receptors (TCRs) that are specific for the recipient alternate polymorphism but not the donor polymorphism.

Previous studies of immunity against solid tumors have revealed that most tumor antigens identified so far are nonmutated self-antigens that are aberrantly expressed in the tumor compared with normal host tissue [4••].

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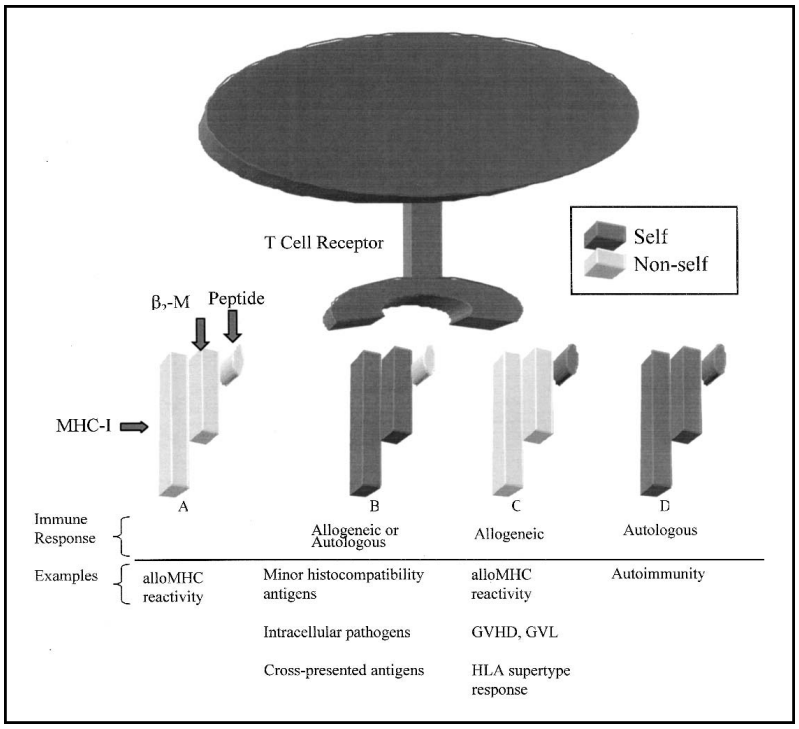
**Figure 1. The spectrum of T-cell autoreactivity and alloreactivity**




The T-cell, pictured on top, engages peptide antigen in the context of MHC on the surface of an antigen-presenting cell. Different types of reactions may result, depending on whether the peptide or MHC are self-derived, allogeneic, or (regarding the peptide) foreign (that is, non-self and non-allogeneic). Published with permission [29].

There are now several such examples in melanoma (MAGE, gp100, tyrosinase) and breast cancer (Her2/*neu*) [5]. An example of an aberrantly expressed tumor antigen in human leukemia is proteinase 3 (Pr3), a 26-kDa neutral serine protease that is stored in primary azurophil granules and is maximally expressed at the promyelocyte stage of myeloid differentiation [6–8]. Pr3 and two other azurophil granule proteins, neutrophil elastase and azurocidin, are coordinately regulated and the transcription factors PU.1 and C/EBPα, which are responsible for normal myeloid differentiation from stem cells to monocytes or granulocytes, are important in mediating their expression [9]. In particular, PU.1 induces expression of the macrophage colony-stimulating factor receptor and the development of monocytes, whereas C/EBPα increases the expression of the granulocyte colony-stimulating factor receptor and leads to mature granulocytes [9,10]. These transcription factors have been implicated in leukemogenesis [10], and Pr3 itself may also be important in maintaining a leukemia phenotype because Pr3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukemia cell line [11].

We have also studied another myeloid-restricted protein, myeloperoxidase (MPO), a heme protein synthesized during very early myeloid differentiation that constitutes the major component of neutrophil azurophilic granules (Table 1). Produced as a single-chain precursor, myeloperoxidase is subsequently cleaved into a light and heavy chain. The mature myeloperoxidase enzyme is composed of two light chains and two heavy chains [12] and produces hypohalous acids central to the microbicidal activity of neutrophil. Importantly, MPO and Pr3 are both over-expressed in a variety of myeloid leukemia cells including 75% of CML patients, approximately 50% of acute myeloid leukemia patients, and approximately 30% of myelodysplastic syndrome patients [13].

**Table 1. Myeloid proteins as potential tissue-restricted leukemia antigens**

| | | mRNA | | |
|---|---|---|---|---|
| Protein | Chromosome | Normal CD34+ | Leukemic CD34+ | Autoimmune syndrome |
| Proteinase 3* | 19p | –/+ | + | Wegener's |
| neutrophil Elastase | 19p | – | + | Wegener's & Vasculitis |
| Myeloperoxidase | 17q22 | + | ++ | Vasculitis |
| Cathepsin G* | 14q11.2 | – | + | Sclerosing cholangitis |

*Naturally processed and presented by CML blasts.
CML, chronic myeloid leukemia.
Data from Barrett *et al.* [29].

What may be critical for our ability to identify T-cell antigens in these proteins is the observation that Pr3 is the target of autoimmune attack in Wegener's granulomatosis [14] and MPO is the target antigen in small vessel vasculitis [12,15,16]. There is evidence for both T-cell and humoral immunity in patients with these diseases. Wegener's granulomatosis is associated with production of cytoplasmic antineutrophil cytoplasmic antibodies with specificity for Pr3 [17], whereas microscopic polyangiitis and Churg-Strauss syndrome are associated with the production of perinuclear ANCA antibodies with specificity for MPO [18,19]. T cells taken from affected individuals proliferate in response to crude extracts from neutrophil granules and to the purified proteins [15,20]. These findings suggest that T-cell responses against these proteins might be relatively easy to elicit *in vitro* using a deductive strategy to identify HLA-restricted peptide epitopes. Based on this hypothesis, we identified PR1, an HLA-A2.1–restricted nonamer derived from Pr3, as a leukemia-associated antigen [21•,22–24] by first searching the length of the protein using the HLA-A2.1 binding motif, the most common HLA allele. Peptides predicted to have high-affinity binding to HLA-A2.1 were synthesized, confirmed to bind, and then used to elicit peptide-specific cytotoxic T lymphocytes (CTL) *in vitro* from healthy donor lymphocytes.

We have found that PR1 can be used to elicit CTL from HLA-A2.1+ normal donors *in vitro*, and that T-cell immunity to PR1 is present in healthy donors and in many patients with CML that are in remission. These PR1-specific CTL show preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow [22]. In addition, PR1-specific CTLs inhibit colony-forming unit granulocyte-macrophage (CFU-GM) from the marrow of CML patients, but not CFU-GM from normal HLA-matched donors [23], suggesting that leukemia progenitors are also targeted.

Using PR1/HLA-A2 tetramers to detect CTL specific for PR1 (PR1-CTL), we found a significant correlation with cytogenetic remission after treatment with interferon-α and the presence of PR1-CTL [21•]. Somewhat surprisingly, PR1-CTLs were also identified in the peripheral blood of some allogeneic transplant recipients who achieved molecular remission and who had converted to 100% donor chimerism. PR1/HLA-A2 tetramer-sorted allogeneic CTL from patients in remission were able to kill CML cells but not normal bone marrow cells in 4-hour cytotoxicity assays, thus demonstrating that the PR1 self-antigen is also recognized by allogeneic CTL [21•]. These studies have established PR1 as a human leukemia-associated antigen, and they established that PR1-specific CTLs contribute to the elimination of CML [21•].

Recently we found another peptide, MY4, a 9-amino-acid peptide derived from MPO that binds to HLA-A2.1, which can be used to elicit CTL from HLA-A2.1+ normal donors *in vitro* [25]. MY4-specific CTLs show preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow [25]. MY4-specific CTLs also inhibit colony-forming unit granulocyte-macrophage (CFU-GM) from the marrow of CML patients but not CFU-GM from normal HLA-matched donors. Like PR1, MY4 is therefore a peptide antigen that can elicit leukemia-specific CTL.

Because of the many striking similarities between immunity to Pr3 and to MPO, it is likely that similar methods applied to the study of immunity against MPO-derived peptides will establish MY4 and other peptides as important leukemia-associated antigens [26]. Using a deductive strategy to uncover potential tumor antigens, we are currently studying sequence data from the human genome project to determine other HLA-restricted epitopes from tissue-restricted antigens. There is a high likelihood that other peptide epitopes can be determined using this approach, especially by focusing on those proteins that are already the known targets of T-cell–mediated autoimmunity.

## T-cell receptor affinity influences GVL

More recently, we have shown that distinct populations of PR1-CTL with either high or low TCR affinity for PR1 can be elicited from PBMC of healthy donors. The high-affinity PR1-CTL cause higher specific lysis of CML cells than low-affinity PR1-CTL. Interestingly, we also found that when high-affinity PR1-CTLs were exposed to target cells that expressed high concentrations of target antigens, the PR1-CTL underwent apoptosis within 18 hours. However, there was no apoptosis when the high-affinity PR1-CTLs were exposed to a 2-log lower concentration of PR1 antigen. Furthermore, we have been unable to either detect or elicit high-affinity PR1-CTL *in vitro* from PBMC of untreated CML patients. Because healthy HLA-A2+ individuals have PR1-CTL with high-affinity TCR, however, this suggests that the high-affinity PR1-CTL may have been deleted during the outgrowth of the leukemia by CML cells that over-express the PR1 tumor antigen.

Taken together these findings suggest that, in addition to HLA disparities and polymorphic mHAs, self-antigens may be the targets of alloreactive T cells. These observations form the basis for a mechanism of alloreactivity and subsequent new treatment strategies based on targeting self-antigens in the allogeneic setting. Specifically, GVL alloreactivity may in part be caused by the transfer from donor to recipient of high-affinity CTL with leukemia self-antigen specificity that were not deleted from the T-cell repertoire during normal T-cell

development in the donor. On this basis, GVL could be separated from GVHD if the target self-antigen expression was limited to hematopoietic tissue only. Further specificity from aberrant expression of the target self-antigen in the leukemia compared with normal hematopoietic cells might give rise to a critical number of recognizable surface peptide epitopes that would surpass the activation threshold of high-affinity T cells, whereas the lower level of antigen expressed in the normal hematopoietic cells would not. This would result in preferential killing and elimination of leukemia cells over normal hematopoietic cells by the transplanted high-affinity donor T cells. As a consequence, residual normal recipient hematopoietic cells would be spared and could then coexist with donor hematopoietic cells after successful elimination of the leukemia, a phenomenon that occurs in some BMT recipients that achieve cytogenetic remission.

Arguing against this hypothesis is the observation that CML recipients of syngeneic stem cell grafts, which have few mHA differences but which should also contain high-affinity PR1-CTL, suffer higher relapse rates than do recipients of allogeneic grafts [27]. However, because high-affinity PR1-CTLs are present at a very low precursor frequency in healthy donors, major and minor histocompatibility antigenic differences may be required to provide generalized heightened immunity via indirect effects mediated by cytokine secretion, which might broadly decrease the threshold of TCR activation and drive the expansion of high-affinity self-antigen–specific T cells. This would also explain the development of GVHD, because this could lead to the uncovering of cryptic antigens and also to epitope spreading [28••]. More effective GVL might therefore be observed after syngeneic BMT if higher numbers of high-affinity CTL were initially transplanted. Consistent with this is the clinical observation that fewer relapses occur in syngeneic graft recipients who receive higher total nucleated cell doses during initial transplant [29], suggesting that an initially high number of high-affinity self-antigen–specific CTL might compensate for their innately low precursor frequency and the absence of significant alloreactivity in this setting.

## Molecular basis of allogeneic GVL against self-antigens

The observation that self-antigens can also be recognized as tumor antigens by allogeneic T cells presents an opportunity to redirect potent alloreactivity toward these self-antigens. Our observations, which are consistent with an evolving overall understanding of the molecular basis of allorecognition, suggest a unique approach to immunotherapy. It has long been recognized that very vigorous T-cell responses occur when donor tissue is transplanted into an MHC-mismatched recipient, where up to 10% of recipient peripheral T cells respond to allo-MHC antigen. This high frequency of recipient-reactive donor T cells occurs because of the increased binding energy of donor TCR to the recipient peptide/allo-MHC combination, and either the peptide or the polymorphic amino acid differences in the allo-MHC may account for this higher binding energy. In addition, either interaction may increase the binding energy relative to that of donor TCR bound to peptide plus donor (self)-MHC. Although it was originally thought that allo-MHC differences accounted for the increased binding energy, Reiser *et al.*, recently showed that T-cell alloreactivity can be caused by more effective interaction of the TCR with both peptide *and* allo-MHC residues [30••].

TCR on the surface of $CD8^+$ CTL recognize short peptides 8 to 11 amino acids long that are derived from intracellular proteins and bind to MHC class I. During normal T-cell maturation, TCRs are selected based on their binding affinity to peptide plus self-MHC, a process referred to as positive selection [31]. Likewise, antigen recognition by alloreactive T cells also depends on peptides within the allo-MHC groove. Most of these T cells exhibit some degree of peptide specificity, and the frequency of peptide-specific alloreactive T cells was recently found to be higher when the allo-MHC was more similar to self-MHC [30]. Thus, polymorphic residues on allo-MHC might give rise to altered amino acids that could raise the binding threshold of the TCR above the interactions produced by shared residues on self-MHC, the latter having been accomplished through positive T-cell selection in the donor. Consequently, an allo-MHC molecule with more extensive polymorphism would have a higher likelihood of losing the energy of interactions gained from positive selection, and T cells that can react productively with these highly polymorphic allo-MHC would be of lower frequency than T cells that have the potential to cross-react with allo-MHC of a lower degree of polymorphism.

For the T cell to become activated, the added TCR interaction with the bound peptide need only raise the affinity slightly above the energy contributed by the TCR interaction with allo-MHC alone. The observation that only small increases in binding energy above the direct contribution by TCR interaction with allo-MHC are necessary to reach threshold for T-cell activation is consistent with the observation that alloreactive T cells, although peptide-dependent, appear to be less peptide-specific than TCR interactions with self. This decreased peptide specificity refers only to T-cell activation, a downstream measure of antigen recognition and cell function. The crystallographic data from Reiser *et al.* is consistent with the likelihood that various peptides, when bound to an allo-MHC, may appear cross-reactive in eliciting T-cell responses because their interactions with the TCR are above the critical threshold of activa-

tion but their individual affinities for the TCR may be different [30]. This has recently been demonstrated in a murine model [32] and in humans, where non-self CTLs maintain specificity for the HA-1 minor histocompatibility antigen across different HLA alleles [33•].

The MHC alleles can differ from one another by as many as 20 amino acids, and most of these polymorphic residues line the peptide binding cleft that determine peptide-binding specificity. Nevertheless, a few polymorphic residues are exposed on the outer surface of the MHC α-helices and hence would be able to interact with the TCR. These might allow alloreactive TCR to adopt an MHC-binding geometry that is similar to the original TCR conformation that contacted self-MHC molecules and resulted in positive T-cell selection. In addition, changes in peptide/MHC shape complementarity might also occur by buried or non-exposed polymorphisms in the MHC that preserve peptide specificity but that may still increase TCR-binding affinity [30].

To better understand how to maximize the full therapeutic potential of alloreactive T cells, we must consider the degeneracy of a single peptide binding to various MHC alleles. Distinct MHC alleles that bind a single common peptide have been termed super-type alleles, and they share similar amino acid residues in their peptide-binding pockets that bind common peptides [34,35]. However, polymorphic residues on the $\alpha_1$ and $\alpha_2$ domains of super-type allo-MHC may also contribute higher binding energies with alloreactive TCR than residues at the same positions on self-MHC. Under this circumstance, the bound peptide would become the common TCR-restricting element, directing the potent effector function of the alloreactive CTL against target cells that express the same common peptide but distinct polymorphic allo-MHC. If the peptide were preferentially expressed in the tissue from which the tumor was derived, it would be transformed into a potent tumor antigen in the context of this alloreactivity.

This model suggests how alloreactivity directed toward tissue-restricted self-peptides might be exploited to take advantage of the vigorous alloreactivity that occurs after MHC-mismatched stem cell transplantation. In the case of the PR1 peptide, for example, we have recently shown that lymphocytes from an HLA-A*0201-positive healthy donor contain a population of T cells with high-affinity PR1-specific TCR. We have also found that PR1 also binds equally well to other alleles in the HLA-A2 super type (Molldrem, unpublished observations, June 2002). Therefore, CTL adoptively transferred from an HLA-A*0201 donor to a HLA-A*0205 CML patient might result in more potent GVL against CML if the TCR of the donor CTL reached activation threshold earlier than residual autologous PR1-CTL similarly exposed to PR1 in the context of HLA-A*0205. Because CTL with high-affinity TCR for the PR1 peptide exist in most healthy donors without evidence of self-hematopoietic tissue destruction, it is reasonable to believe that normal HLA-A*0205 hematopoietic cells expressing normal levels of PR1 also would not be recognized by the alloreactive HLA-A*0201 CTL with higher TCR affinity for HLA-A*0205. This would facilitate the development of hematopoietic microchimerism in the transplant recipient with both HLA-A*0205 and HLA-A*0201 hematopoietic cells.

## Advantages and disadvantages of self-antigens as GVL targets

Several authors have suggested that one way to enhance GVL and reduce GVHD would be to adoptively transfer antigen-specific T cells from the donor to the recipient [36••,37]. Adoptive transfer to BMT recipients of alloreactive T cells with specificity for self-peptides after an initial T-cell–depleted MHC-mismatched transplant offers several potential advantages over strategies utilizing precise HLA and possible mHA matching to reduce the incidence of GVHD. First, it would greatly expand the number of potential donors for allogeneic stem cell transplantation, which is the largest obstacle to extending this potentially curative treatment modality to more patients. Donor-recipient pairs that shared a common HLA supertype would be sufficient. Second, the time required to expand peptide antigen-specific CTL *ex vivo* for adoptive transfer to recipients to induce GVL might be eliminated or greatly reduced because of the high initial precursor frequency of the alloreactive CTL. Third, because the target peptide is a self-antigen, it would eliminate the need to find tissue-restricted mHA differences between donor and recipient if mHA-specific CTL were to be adoptively transferred to the recipient to induce GVL reactivity.

This strategy of self-peptide–directed alloreactivity might also be applied to the treatment of solid tumors, where many self-antigens have already been discovered but where effective autologous immune responses are lacking [38]. It also suggests a possible future strategy for the treatment of autoimmune diseases if suitable peptide antigens could be identified and their gene expression was restricted to T cells or even to hematopoietic cells.

## Conclusions

Obstacles to this approach remain, however. We must

(1) determine the key MHC residues that are involved in positive selection;
(2) identify certain tissue-restricted self-peptides that are recognized by T cells; and
(3) determine which of those peptides also bind to different alleles that are confined to a given HLA super type.

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