Allorestricted cytotoxic T cells specific for human CD45 show potent antileukemic activity


Recent advances have made haploidentical transplantation for leukemia feasible, but the rigorous T-cell depletion used contributes to the high relapse rates observed. We have attempted to improve the graft-versus-leukemia (GVL) effect by generating allorestricted cytotoxic T lymphocytes (CTLs) directed against human CD45. Such CTLs should recognize patient hematopoietic cells including leukemia, enhancing donor cell engraftment and improving the GVL effect, but they should not recognize host nonhematopoietic tissues or donor cells from the graft. Using the T2 binding assay, 4 CD45-derived peptides were found to bind HLA-A2 molecules. These peptides were used to generate cytotoxic T-cell lines from HLA-A2+ donors by sequential stimulation with peptide-pulsed HLA-A2+ stimulators, and the lines obtained were screened for peptide-specific cytotoxicity. Using one of these peptides (P1218), it was possible to generate peptide-specific, allorestricted CTLs in 3 of 7 responders. P1218-specific CTL lines show potent cytotoxicity against hematopoietic cell lines coexpressing HLA-A2 and CD45 but not CD45 loss variants. Studies with stable transfectants of 293 cells demonstrated recognition by P1218-specific CTLs of endogenously expressed CD45. Likewise P1218-specific CTLs recognized peripheral blood mononuclear cells (PBMCs) from HLA-A2+ patients with chronic myeloid leukemia (CML) and leukemic blasts in HLA-A2+ patients with acute myeloid leukemia (AML), but they were unable to lyse HLA-A2+ fibroblasts or HLA-A2− normal PBMCs. Coculture of CD34+ PBMCs and bone marrow mononuclear cells (BMMCs) with P1218-specific CTL significantly inhibited colony-forming unit–granulocyte macrophage (CFU-GM) formation in HLA-A2+ healthy controls and CML patients but resulted in no significant inhibition in HLA-A2− healthy controls. These studies demonstrate that P1218-specific cytotoxic T lymphocytes (CTLs) have potent activity against leukemic progenitors and suggest that adoptive immunotherapy with allorestricted CTLs directed against CD45 epitopes may be useful in restoring the GVL effect after HLA-A2−-mismatched haploidentical transplantation. Further, because P1218-specific CTLs also recognize healthy HLA-A2+ progenitors, such CTLs could also contribute to host myelobloliation and enhance donor cell engraftment.

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target molecule for allorestricted CTLs in patients with HLA-A0201 mismatch, and we have demonstrated that such CTLs show significant antileukemic activity in vitro.

Materials and methods

Cell lines, generation of primary fibroblasts, and purification of CD34+ cells

The T2 cell line is transporter-associated with antigen processing (TAP) deficient, resulting in inefficient loading of human leukocyte antigen class I molecules with endogenous peptides.15 As a consequence, the HLA-A0201 molecules of T2 cells can be efficiently loaded with exogenous peptides. *Drosophila* cells transfected with HLA-A0201 under the control of a metallothionein-inducible promoter, human β-2 microglobulin, B7.1, and intracellular adhesion molecule 1 (ICAM-1) were the kind gift of Dr M. Jackson. RMA/S-A2 is a mouse lymphoma line transfected with a human genomic HLA-A0201 clone including the endogenous promoter. C1R-A2 is an HLA-A0201–transfected Epstein-Barr virus (EBV)–transformed lymphoblastoid cell line, and WS29 cell is an EBV-transformed lymphoblastoid cell line from an HLA-A0201–negative patient. BV173 is an HLA-A0201–positive cell line derived from a patient with CML in lymphoid blast crisis that lost expression of CD45. HeLa-A2 cells were made by transfecting the human cervical epithelial cell line HeLa with a genomic HLA-A0201 clone, including the endogenous promoter. T4D7 and MCF-7 are HLA-A2+ and HLA-A2+ breast cancer epithelial cell lines, respectively. U-266 is a myeloma cell line known to be biphenotypic with regard to CD45 expression.16 This cell line was sorted into CD45− and CD45+ fractions using a FACScan flow cytometer (BD Biosciences, San Jose, CA). Stable transfectants of 293 cells (a human embryonic kidney cell line) expressing CD45RO were made using a transient retroviral supernatant as described in the report by Kelly et al.17 Briefly, the pZIP/CLA1 plasmid16 expressing CD45RO in pZIPNeo was cotransfected with the plasmids pSG-PAM3 (containing the Moloney murine leukemia virus gag and pol genes) and pRD-114-env (containing the env gene from the feline leukemia virus) into 293T cells. Forty-eight hours later the supernatant from these cells was used to transduce wild-type 293 cells with 8 µg/mL polybrene (Specialty Media, Phillipsburg, NJ). After another 48 hours, cells were placed under selection with 750 µg/mL G418. Initial experiments with 2 constructs carrying full-length CD45RA yielded neomycin-resistant cells, but CD45 expression was not detected, suggesting this gene may be toxic in some nonhematopoietic cells. In neomycin-resistant 293 cells stably transfected with pZIP/CLA1, approximately 20% of cells expressed CD45. These CD45+ cells were isolated using a FACScan flow cytometer (BD Biosciences) and maintained expression of this molecule in culture. Primary fibroblast cultures were established by culture of fragments of a fresh 4-mm skin biopsy specimen under coverslips in Dulbecco modified Eagle medium (DMEM) supplemented with 20% fetal calf serum in 6-well plates, followed by passages into flasks. In some experiments, fibroblasts were cultured with 250 IU/mL interferon-γ (IFN-γ) for 48 hours before cytotoxicity assays to increase the expression of HLA class 1 and 2 molecules. Fibroblasts were harvested for cytotoxicity assays using enzyme-free cell-dissociation buffer (Gibco-BRL, Paisley, United Kingdom). CD45+ peripheral blood mononuclear cells (PBMCs) or bone marrow mononuclear cells (BMMCs) were purified using the MiniMACS system according to manufacturer’s instructions (Miltenyi Biotech, Bisley, United Kingdom) and had a purity of more than 90% as assessed by fluorescence-associated cell sorter (FACS) analysis.

Synthetic peptides, peptide-binding assays, and intracytoplasmic flow cytometry

Using the BIMAS computer model (http://bimas.dct.nihs.gov/), nonameric and decameric peptides from the human CD45 sequence were ranked according to their predicted ability to bind HLA-A0201. Peptides derived from human CD45 and a control HLA-A0201 binding peptide derived from...
the core antigen of hepatitis B virus (residues 18-27, FLPSDFFPSV) were synthesized using fluoronylmethoxycarbonyl chemistry. The quality of the peptides was assessed by high-performance liquid chromatography analysis, and the expected molecular weight was observed using matrix-assisted laser desorption mass spectrometry. Peptides were dissolved in phosphate-buffered saline (PBS; pH 7.4) to give a concentration of 2 mM. Peptide-binding assays were performed by overnight incubation of T2 cells (10^5 cells/well) in 96-well plates with serial dilutions of peptides in RPMI 1640 with 10% boiled fetal calf serum (to prevent protease activity), followed by FACS analysis for surface expression of HLA-A0201. Mean fluorescence intensities at varying concentrations of peptide were compared.

Intracytoplasmic flow cytometry for IFN-γ was performed using the FastImmune intracellular cytokine detection assay (Becton Dickinson, San Jose, CA) according to manufacturer’s instructions. Briefly, CTLs were stimulated with peptide-loaded T2 cells or 25 ng/mL phorbol 12-myristate 13-acetate (PMA)/1 μM ionomycin for 6 hours. Then 10 μg/mL brefeldin A was added for the final 4 hours of coculture. Following this, cells were washed and stained with surface antibodies to CD4 or CD8 (PerCP conjugates) and CD69 (phycoerythrin [PE] conjugate) to ensure activation. Cells were then fixed, permeabilized, and stained with an anti–IFN-γ antibody (fluorescein isothiocyanate [FITC] conjugate) and were analyzed by 3-color flow cytometry.

Generation of allo-HLA-restricted CTL lines
PBMCs were separated from buffy-coat packs using Ficoll-Hypaque density gradient centrifugation and were stained with monoclonal antibodies HB54 (anti–HLA-A2, B17) and HB117 (anti–HLA-A2, A28). A2-negative PBMCs were used as responders. T2 cells that had been loaded with 100 μM peptide for 6 hours and then irradiated (60 Gy) were used as initial stimulators. Each well of a 24-well plate received 2 × 10^5 responder PBMCs and 2 × 10^5 irradiated, peptide-loaded T2 stimulators in 2 mL RF10 (RPMI with 10% fetal calf serum) with 2.5 ng/mL recombinant human IL-7 (huIL-7) and 500 nM peptide. Drosophila A2 cells were used for the initial restimulation. These were induced in 100 mM CuSO_4 for 48 hours, washed 3 times with medium, and loaded with 100 μM peptide for 4 hours. On day 6, T cells were harvested, plated at a density of 5 × 10^5 per well, and restimulated with 2 × 10^5 peptide-coated Drosophila A2 cells with the addition of 2 × 10^5 autologous irradiated (30 Gy) PBMCs as feeders, in fresh medium containing 10% QS4120 culture supernatant (containing anti-CD4 antibodies), 10 U/mL recombinant huIL-2 (Roche Diagnostics, Lewes, United Kingdom), 2.5 ng/mL recombinant human IL-7 (huIL-7) and 500 nM peptide. On day 18, T cells were harvested and restimulated in a similar fashion but using RMA/S-A2 cells that had been incubated at 25°C for 48 hours, washed 3 times with medium, and loaded with 100 μM peptide for 4 hours. On day 6, T cells were harvested, plated at a density of 5 × 10^5 per well, and restimulated with 2 × 10^5 peptide-coated irradiated peptide-coated irradiated T2 cells and 2 × 10^5 irradiated HLA-A2–negative PBMC feeders were added to each well in RF10 with 10 U/mL huIL-2 and 2.5 ng/mL huIL-7. Cultures received additional stimulator and feeder cells on day 40. Six days later, the cytotoxicity of each well was tested against T2 target cells coated with the immunizing peptide or a control HLA-A0201–binding peptide. Peptide-specific microcultures were expanded and restimulated weekly in 24-well plates by adding 2 × 10^4 irradiated feeders and 2 × 10^6 peptide-loaded, irradiated T2 cells as stimulators in medium containing 10 U/mL IL-2 and 2.5 ng/mL IL-7.

Cytotoxicity and colony-inhibition assays
Cytotoxic T-lymphocyte assays were performed as described. Briefly, 3 × 10^5 targets were labeled with 100 μCi (3.7 MBq) chromium Cr 51 for 2 hours, washed 3 times, and added to serial dilutions of effector cells in triplicate, round-bottomed, 96-well plates to obtain a total volume of 200 μL/well. In some experiments T2 cells were peptide loaded by preincubation with 100 μM synthetic peptide before labeling. In antibody-blocking experiments, 10 μL chromium-labeled target cells were preincubated with 10 μL blocking antibodies to HLA class 1 (clone W6/32; DAKO, Carpinteria, CA), HLA class 2 (clone CR3/43; DAKO), or isotype control for 30 minutes at 37°C before the addition of CTLs. In some experiments a 50-fold excess of cold K562 cells or a 20-fold excess of peptide-loaded T2 cells was added to cultures to assess specificity. Assay plates were incubated for 6 hours at 37°C, 5% CO_2, and 100 μL supernatant was harvested and counted using a Wallac gamma counter (Wallac, Milton Keynes, United Kingdom). Specific lysis was calculated by the equation (experimental release − spontaneous release)/maximum release − spontaneous release) × 100%. Colony-inhibition assays were performed by culturing CD34+ PBMCs and BMMCs in the presence and absence of CTLs at an effector-target ratio of 10:1 for 6 hours. CTL-treated and mock-treated CD34+ cells were then plated in duplicate plates in methylcellulose supplemented with 100 ng/mL granulocyte-colony-stimulating factor (G-CSF), 1 ng/mL granulocyte macrophage–colony-stimulating factor (GM-CSF), 5 ng/mL recombinant human IL-3 (rhIL-3), and 20 ng/mL recombinant human stem cell factor (rhSCF). Granulocyte macrophage-colony-forming units (CFU-GM) with more than 100 cells were counted after 10 to 12 days. Percentage inhibition of colony formation was determined as (no. CFU-GM in the presence of CTL/no. CFU-GM in the absence of CTL) × 100%.

Results
Identification of CD45 peptides binding HLA A201 and generation of CD45-specific CTLs
As shown in Figure 2A, we confirmed high levels of CD45 expression on leukemic blasts from patients with acute lymphoblastic leukemia (ALL) and AML and on PBMCs from patients with CML, indicating that CTLs directed against CD45 are likely to have activity against myeloid and lymphoid leukemias. The level of expression was higher in normal and CML PBMCs than in ALL and ALL blasts. To determine the expression pattern of CD45 in a variety of human tissues, we performed Northern blot analysis. Figure 2B shows that the expression of CD45 is restricted to hematopoietic tissues, with high levels of mRNA expression in the thymus, spleen, and peripheral blood. The apparent low level of

Figure 2. CD45 is expressed on leukemic blasts and is hematopoiesis specific. (A) FACS analysis showing surface CD45 expression in PBMCs from 5 healthy controls compared with 5 patients with CML and BMMCs from 5 patients with AML or ALL. Each sample was stained with isotype control (dotted lines) and anti-CD45 (solid lines) antibodies, and the corresponding histograms are shown. (B) Northern blot analysis of CD45 RNA expression in various tissues.
CD45 RNA seen in heart and liver probably resulted from the presence of contaminating hematopoietic cells. To identify CTL epitopes in CD45, we synthesized 16 peptides that were predicted by the BIMAS computer model to bind to HLA-A0201. We investigated the relative ability of these 16 peptides to bind HLA-A0201 by assaying the ability of exogenously pulsed peptide to up-regulate HLA-A0201 expression on the cell surface in the TAP-deficient cell line T2. Figure 3 shows that 4 peptides (P292, P684, P737, P1218) showed significant binding to HLA-A0201, whereas the remaining peptides did not bind.

We then attempted to generate allorestricted CTLs with 4 of these peptides by stimulation of PBMCs from healthy HLA A0201–negative responders with a variety of HLA A0201+ APCs exogenously pulsed with peptide, followed by limiting-dilution cultures to isolate peptide-specific CTL lines. No peptide-specific lines were generated using P292, P684, or P737. However, peptide-specific CTL lines directed against P1218 were generated from 3 of 7 donors. This peptide (sequence FLYDVIAST) is located in the second phosphotyrosine phosphatase domain of the cytoplasmic tail of CD45 and was the best binder to HLA A0201 in peptide-binding assays. As illustrated in Figure 4A, all 3 of these CTL lines showed exquisite peptide specificity in cytotoxicity assays, with almost total killing of T2 targets pulsed with P1218 at low effector-target ratios and little cytotoxicity against T2 cells alone or pulsed with a control peptide known to bind HLA A0201.

Little major histocompatibility complex (MHC)–unrestricted cytotoxicity against K562 cells was observed, and cytotoxicity against T2 cells pulsed with P1218 was not abrogated by a 50-fold excess of cold K562 cells (Figure 4C), demonstrating the observed cytotoxicity was specific. All 3 CTL lines were initially 60% to 70% CD8+ and 30% to 40% CD4+, but with prolonged culture CD8+ cells increased to 90% to 95% and the CD4+ population decreased to 3% to 5%. To test the avidity of our T-cell lines, we performed cytotoxicity assays against T2 targets titrating the concentration of exogenously pulsed P1218. As shown in Figure 4B, line 2 showed the highest avidity with significant cytotoxicity at nanomolar levels of peptide. Line 2 was therefore selected for further experiments.

To determine whether the response against P1218 peptide-pulsed T2 cells was mediated by CD4 or CD8+ cells, intracytoplasmic IFN-γ flow cytometry was used to study the expression of IFN-γ after the exposure of P1218-specific CTLs to peptide-pulsed T2 cells. As illustrated in Figure 5, 28.4% of CD8+ cells from line 2 expressed IFN-γ in response to T2 cells pulsed with P1218, but only 2.5% expressed IFN-γ in response to T2 cells pulsed with control peptide. This demonstrates peptide-specific secretion of IFN-γ by the CD8+ population within the CTL line. In contrast, there was no secretion of IFN-γ in response to either peptide by CD4+ cells (data not shown).

**CD45-specific CTLs kill leukemic cell lines**

To determine whether P1218-specific CTLs were able to recognize endogenously processed CD45, we analyzed cytotoxicity against a panel of leukemic cell lines. As can be seen in Figure 6, P1218-specific CTLs were able to efficiently lyse the B-lymphoblastoid cell line, CIR-A2, which expresses CD45 and
were unable to lyse BV173, a leukemia cell line that expresses HLA-A0201 but has lost expression of CD45 or 293 cells, an embryonic kidney cell line expressing HLA-A0201. These results demonstrate that HLA-A0201 expression alone is insufficient for recognition by this CTL line and suggest that coexpression of CD45 is required. Surprisingly, at high effector-target ratios, 2 nonhematopoietic targets, MCF-7, an HLA-A0201⁺ breast cancer cell line, and HeLa-A2, a cervical epithelial cell line transfected with HLA-A0201, were also killed by P1218-specific CTLs (though less well than C1R-A2 targets), despite the fact that neither of these cell lines expressed detectable CD45 by FACS staining. The cytotoxicity of P1218-specific CTL against both these targets was only partially inhibited by a 50-fold excess of cold K562 cells (data not shown), indicating that recognition of these HLA-A0201⁺, CD45⁻ targets is not primarily caused by NK activity.

The observed cytotoxicity against CD45⁻ targets could be attributed to cross-recognition of other HLA-A0201/peptide complexes by CD45-specific CTLs or to the presence of a different A0201-alloreactive clone in the CTL line. To address this, the killing of HLA-A0201⁺, CD45⁻ targets was analyzed in the presence of an excess of cold T2 target cells loaded with the P1218 peptide or of an irrelevant HLA-A0201–binding peptide derived from hepatitis B virus. Figure 6B shows that at a low effector-target ratio (5:1), the P1218-loaded cold targets completely inhibited killing of the HLA-A0201⁺, CD45⁻ targets, whereas inhibition was incomplete at high (30:1) CTL numbers. Conversely, cold T2 targets presenting the irrelevant hepatitis B virus (HBV) peptide did not inhibit killing at a low effector-target ratio but showed some inhibition at high CTL numbers. These data suggest that the CD45-specific CTL line contains a small number of CTLs recognizing HLA-A0201 molecules presenting irrelevant peptide epitopes. This is consistent with the observation that subcloning of the CTL line gave rise to short-term CTL clones that displayed P1218 specificity and killed C1R-A2 cells, but not the nonhematopoietic HeLa-A2 cells (not shown). We were unable to expand and maintain these subclones, which probably reflects an important function of CD4 T cells in promoting long-term CTL growth, as has previously been shown in vivo.17,18

**P1218-specific CTLs recognize endogenously presented CD45 in malignant hematopoietic cell lines**

To determine whether P1218-specific CTLs are capable of recognizing endogenously processed CD45 in malignant hematopoietic cells, we took advantage of the observation that the myeloma cell line U-266 is...
observed against the CD45 for recognition of U-266 cells by the CTLs. The low-level cytotoxicity results demonstrate that endogenously expressed CD45 was sufficient for recognition of CD45-negative targets, cytoplasmic CD45 was lysis. These results demonstrate that endogenously expressed CD45 was sufficient for recognition of U-266 targets, and the low-level cytotoxicity observed against the CD45-negative U-266 cells was significant. These results demonstrate that endogenously expressed CD45 was sufficient for recognition of U-266 cells by the CTLs. The low-level cytotoxicity observed against the CD45-negative U-266 cells was significant. These results demonstrate that endogenously expressed CD45 was sufficient for recognition of U-266 targets, cytoplasmic CD45 was lysis.

To further show that P1218-specific CTLs recognize endogenously expressed CD45, we generated a stable transfectant of the HMV-A0201 kidney cell line, 293, expressing CD45RO (Figure 7Aii,iv). As shown in Figure 7Bi-iv, wild-type 293 cells were killed poorly, whereas after the introduction of CD45 this same cell line was efficiently lysed by P1218-specific CTLs, demonstrating that these CTLs recognized endogenously expressed CD45.

### CD45-specific CTLs are cytotoxic to primary normal hematopoietic and leukemic cells

To analyze the activity of P1218-specific CTLs against primary hematopoietic targets, cytotoxicity assays were performed using CML PBMCs and leukemic blasts from HLA-A0201–positive patients with AML. As illustrated in Table 1, P1218-specific CTLs showed significant cytotoxicity against PBMCs from 4 of 4 patients with CML and against leukemic blasts from 4 of 4 patients with AML tested. No killing of HLA-A0201 normal or CML PBMCs was observed, demonstrating that the observed cytotoxicity is HLA-A2 restricted. Leukemic blasts from 2 HLA-A0201+ patients with ALL, both of which expressed CD45, were not killed (data not shown).

To determine tissue specificity of P1218-specific CTLs against primary targets, we performed cytotoxicity assays using primary fibroblasts from HLA-A0201+ healthy donors as targets. After induction with IFN-γ, fibroblasts showed comparable levels of HLA class 1 expression to PBMCs, as assessed by FACS analysis (data not shown). As can be seen in Table 1, P1218-specific CTLs showed no cytotoxicity against HLA-A2+ fibroblasts, suggesting that P1218-specific CTLs kill in a hematopoiesis-specific manner. In contrast, fibroblasts pulsed with exogenous P1218 were lysed, demonstrating that the lack of cytotoxicity against unpulsed fibroblasts was caused by the absence of the cognate antigen.

### Discussion

We have investigated a novel approach to adoptive immunotherapy to restore the GVL effect following haploidentical HSCT. Rather
than relying on a disparity in antigenic expression between host and donor, we have exploited an HLA mismatch to generate CTLs directed against a hematopoietic antigen common to host and donor, recognized only in the context of host HLA molecules. Because it circumvents immunologic tolerance, this allorestricted approach is suitable for raising CTLs against any hematopoietic antigen. CD45 was chosen as a candidate because of its hematopoietic-restricted expression and because it is abundantly expressed in myeloid and lymphoid leukemia. The described P1218 epitope is located on the C-terminal second phosphotyrosine phosphatase domain and is not known to be polymorphic or to function as a minor histocompatibility antigen in patients with HLA match. It is anticipated that autologous and HLA-matched CTLs are tolerant of this epitope. The allorestricted CTL lines generated in this study contained predominantly CD8+ cells, particularly after prolonged culture, though some CD4+ cells persisted in all lines established. In previous experiments with allorestricted CTL lines directed against WT-1, CD4+ cells were also present and showed no proliferative response to peptide or T2 cells but did show strong interleukin-2 (IL-2)--dependent proliferation in the absence of antigen (H.J.S., unpublished data, 2000). Subcloning of these CTL lines yielded CD8+ CTL clones that showed the killing specificity displayed by the line, but the life span of these clones was limited to 6 to 8 weeks in the absence of CD4+ T cells. Similarly, we were unable to expand and maintain subclones of the P1218-specific CTL line. This in vitro observation is similar to what has been observed in clinical trials of adoptive immunotherapy with antiviral CTLs, suggesting that the presence of helper T cells may increase the persistence of infused CTLs.17,18

Our observations that lysis of cell line targets, including U-266, by P1218-specific CTL requires coexpression of HLA-A0201 and CD45, together with our data from stable transfectants of 293 cells, show that this CTL line is capable of recognizing endogenously expressed CD45. Formal demonstration of endogenous presentation of the P1218 epitope, however, will require peptide elution from HLA-A0201+ hematopoietic cells. The activity of P1218-specific CTLs against the HLA-A0201+ nonhematopoietic cell lines HeLa-A2 and MCF-7 in the absence of CD45 has been explored in cold-target inhibition experiments. Results suggest that the unexpected killing activity is not caused by MHC-unrestricted NK activity but rather is most likely caused by the presence of a small number of alloreactive CTLs capable of recognizing A0201 molecules presenting irrelevant peptide epitopes. It is interesting to note that though our CTL line showed significant killing of TAP-deficient T2 cells without peptide pulsing (Figure 2A), and of HeLa-A2 and MCF-7 cells (Figure 6A), little killing was observed against other A0201-positive targets such as BV173, 293, and IFN-γ-induced primary fibroblasts (Figure 6A, Table 1). This suggests that allreactivity is not directed against HLA-A0201 molecules per se but involves a peptide, or a set of peptides, presented by T2, HeLa-A2, and MCF-7. Interestingly, using a T2 stimulation protocol similar to the one used here, Moris et al19 have recently demonstrated the presence of alloreactive CTL capable of recognizing a limited set of peptides presented by HLA-A0201.

Allorestricted CTLs directed against P1218 show significant cytotoxicity against primary leukemic targets from HLA-A0201+ patients with AML and CML. Leukemic blasts from 2 HLA-A0201+ patients with ALL were not lysed, and further studies will be needed to determine whether this is generally true in ALL. In this regard it is interesting to note that ALL blasts are also less susceptible to lysis by alloreactive NK clones, perhaps because of their lack of LFA-1 expression,20 and they also appear resistant to anti-CD45 antibody-mediated lysis (M.K.B., personal communication, June 2002). Additionally, P1218-specific CTLs strongly inhibit colony formation from CD34+ CML progenitors in an HLA-A0201-restricted fashion. These results suggest that P1218-specific CTLs may have significant antileukemic activity in the context of an HLA-A0201+ patient undergoing transplantation from an HLA-A0201− donor. However, the extent to which our in vitro data will correlate with in vivo antileukemic activity is unknown. In this regard, it will be necessary to determine whether P1218-specific CTLs can eliminate leukemic stem cells in the context of SCID/NOD mice, as has been shown with CTLs directed against major histocompatibility antigens.21 The lack of cytotoxicity of P1218-specific CTLs against HLA 0201+ fibroblasts suggests that, as expected, such CTLs do not recognize primary nonhematopoietic targets and, therefore, are unlikely to cause GVHD, provided that CTL therapy is used in patients with high levels of donor chimerism who undergo transplantation. Recent murine studies have clearly indicated that antigen presentation by host APCs plays a key role in triggering GVHD.22 Similarly, the absence of activity of P1218-specific CTLs against HLA-A2− normal PBMCs suggests that, by virtue of their HLA restriction, such CTLs would not adversely affect donor-derived cells from the graft. These data suggest that allorestricted CTLs directed against CD45 epitopes may provide a useful novel reagent in restoring the GV effect after haploidentical HSCT. Further, in contrast to allorestricted CTLs directed against WT-1,10 our colony-inhibition assays demonstrate that P1218-specific CTLs also recognize healthy HLA-A0201− progenitors. Such CTLs potentially contribute to myeloablation as an immunologic conditioning agent.

Sufficient numbers of P1218-specific CTLs can be generated for clinical protocols using our current methodology; once the CTL lines are established, they are easily expanded. Clearly, however, if such an approach is to be adapted to the clinical setting, our protocol for the initial generation of allorestricted CTLs will have to be simplified greatly. Selection of P1218-specific CTLs from bulk cultures with HLA-A0201 tetramers loaded with P1218 peptide may allow rapid isolation of peptide-specific CTLs without resorting to cloning. Alternatively, T-cell receptor gene transfer from existing CTL lines could be used to redirect the specificity of circulating T cells, obviating the need to generate allorestricted CTLs for each patient.23

References

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