Bone Marrow Transplant Recipients Have Defective MHC-Unrestricted Cytotoxic Responses Against Cytomegalovirus in Comparison With Epstein-Barr Virus: The Importance of Target Cell Expression of Lymphocyte Function–Associated Antigen 1 (LFA1)


Cytomegalovirus (CMV) remains the most common single infective cause of death following allogeneic bone marrow transplantation (BMT) from major histocompatibility complex (MHC)-identical siblings, whereas Epstein-Barr virus (EBV)-related disease is infrequent. We show here that MHC-unrestricted cytotoxic effector cells in the peripheral blood of BMT recipients are highly effective at killing EBV-infected target cells, but are inactive against CMV-infected target cells. Differential cytotoxicity is associated with disparate target structure expression. Although both EBV- and CMV-infected target cells express viral antigens, it is only those infected with EBV that express the adhesion molecule lymphocyte function–associated antigen 1 (LFA1; CD11a/18). Thus, EBV-infected target cells are able to interact with the principal LFA1 ligand, intercellular adhesion molecule 1 (ICAM1; CD54), which is expressed on posttransplant peripheral blood mononuclear (PBM) effector cells. CMV-infected target cells cannot utilize this ligand. Posttransplant cytotoxicity against EBV-infected target cells is abolished by target and effector cell blockade with monoclonal antibodies (MoAbs) to LFA1 and ICAM1, respectively, demonstrating the functional relevance of this additional ligand interaction. These results provide an illustration both of the importance and of the limitations of MHC-unrestricted cytotoxicity in vivo and may explain the frequency of CMV disease and the relative rarity of EBV-related disease following allogeneic transplantation from MHC-matched siblings. The increased immunosuppression used following MHC-mismatched/matched unrelated-donor BMT may cause this MHC-unrestricted defense mechanism to fail and may contribute to the greatly increased incidence of EBV lymphoproliferative syndrome in these patients.

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expression were associated with enhanced formation of effector/target cell conjugates, enhanced killing of NK-sensitive target cells and the induction of cytotoxicity for previously NK-resistant target cells. Intercellular adhesion molecule 1 (ICAM1; CD54) is a membrane-bound glycoprotein which is weakly expressed on resting lymphocytes, but strongly expressed on activated lymphocytes, and which is functionally important in mediating cellular cytotoxicity of target cells. Lympocyte function-associated antigen 1 (LFA1; CD11a/18) is the principal ligand of ICAM1 and absence of cell surface LFA1 has been proposed as a mechanism of escape from immunosurveillance. Furthermore, the principal ligand, ICAM1, may be particularly important in interactions with virally infected target cells, since lack of induction of cell adhesion molecules ICAM1 and LFA3 has recently been proposed to explain the finding that EBV-positive Burkitt's lymphoma cell lines are not recognized by virus-specific T-cell surveillance in assays where EBV-infected LCL derived from normal B cells of the same patients are clearly recognized.

We provide evidence here of differential MHC-unrestricted cytotoxic sensitivity post-BMT between cells infected with the two herpesviruses, CMV and EBV. We show how this difference is related to target cell surface expression of adhesion molecules and we suggest that this disparity may explain the different pathogenicity of the two viruses following BMT.

MATERIALS AND METHODS

Patient details. Eighteen recipients of BMTs for hematological malignancies and their normal donors were studied. Thirteen patients received HLA-matched sibling allografts and five received autografts. Seven patients were transplanted for acute myeloid leukemia (AML), five for common acute lymphoblastic leukemia (c-ALL), four for T-cell acute lymphoblastic leukemia (T-ALL), one for B-cell acute lymphoblastic leukemia (B-ALL), and one for ALL of undetermined subtype. Their ages ranged from 10 to 44 years (mean, 26). Recipients were conditioned with cyclophosphamide 60 mg/kg twice daily for 3 days if they were in second remission. In patients in first remission, or plus mesna, if they were in first remission, or with cyclophosphamide 50 mg/kg × 2 plus mesna, if they were in first remission, or with cyclophosphamide 45 mg/kg × 2 and cytosine arabinoside 2 g/m² twice daily for 3 days if they were in second remission. In addition, all patients received single-dose total body irradiation at a maximum prescribed dose of 750 cGy to the lungs at a dose rate of 15 ± 2 cGy/min. Allograft patients received HLA-identical sibling marrow depleted of T cells in vitro by specific antibody followed by complement-mediated lysis. The mean T-cell depletion was 96.2%, and the mean number of T cells infused was 7.1 × 10⁹/kg. No routine posttransplant immunosuppression was administered. For autograft patients, cryopreserved autologous marrow was purged in vitro with a combination of CD10 and CD19 antibodies for patients with c-ALL, with CD7 antibody for patients with T-ALL, and with CD19 antibody for the patient with B-ALL. The incidence of graft-versus-host disease (GVHD) in all was low, as previously reported with T-cell-depleted allografts and only a single allograft recipient had acute GVHD (grade II) at the time of study. Three allograft recipients had mild and limited chronic GVHD of skin at the time of study. All had been preceded by acute GVHD, grades I, II, and III, respectively. Therapy for GVHD was with steroids: methylprednisolone at 1 g, rapidly reducing for acute GVHD, and at a dose of 1 to 1.5 mg/kg for chronic GVHD. However, none of the recipients with chronic GVHD were on treatment at the time of study.

Eight of the allograft recipients were seropositive for CMV and five were seronegative. Of the allograft donors, eight were seropositive and five seronegative. All five autograft recipients were seropositive. Two allograft recipients in the study developed CMV disease from which they both died. One patient had pneumonitis at the time of study, which proved rapidly fatal and which was shown to be due to CMV at postmortem examination. A further allograft recipient, who was well at the time of study, developed a fulminating fatal CMV hepatitis 1 month later.

Fibroblast culture. Blastic bone marrow was harvested under sterile conditions into heparin (10 U/mL) from recipients prior to conditioning and from their normal donors on the day of transplant. Marrow was diluted 1:2 with McCoy's 5A medium (Flow Laboratories, Rickmanowsh, Herts, UK) containing 2.20 g/L sodium bicarbonate supplemented with L-glutamine (2 mmol) and penicillin/streptomycin (100 U/mL) and layered in 10-mL aliquots on 10 mL Lympophrep (Nyggaard, Oslo, Norway). After centrifugation at 400 × g for 30 minutes, interface cells were washed and resuspended at 2 × 10⁶ cells/mL in McCoy's 5A medium supplemented as above containing 10% fetal calf serum (FCS) screened for optimal growth of fibroblast cell lines. Cells were incubated in tissue culture flasks at 37°C in 5% CO₂. After 24 hours, the medium was decanted to discard nonadherent cells and replaced with fresh medium. Flasks were incubated to confluence (1 to 3 weeks), after which the cell layer was passed by trypsinization. All adherent cells used in assays were between first and third passage.

Immunohistochemistry of marrow adherent cell layer. Aliquots of passaged adherent cells were grown onto glass slides to confluence and fixed in formalin for 30 minutes. An avidin-biotin complex (ABC) immunoperoxidase technique was performed with hematoxylin counterstain using mouse monoclonal antibodies (MoAbs). MoAbs used were (1) Vimentin (Dako, Glostrup, Denmark), 57-Kd intermediate filament protein expressed on fibroblasts and endothelial cells; (2) Mac-387 (Dako), cytoplasmic antigen expressed on monocytes, macrophages, and tissue histiocytes; and (3) VWF, von Willebrand factor (factor VIII-related antigen) expressed on endothelial cells (kind gift of Dr Alison Goodall). Endogenous peroxidase was inhibited with 0.5% H₂O₂ in methanol for 10 minutes and washed under tap water. Following a subsequent wash in Tris-buffered saline (TBS), slides were incubated at room temperature with the primary antibody diluted between 1:10 and 1:20 for 30 minutes. Following washing, biotinylated rabbit anti-mouse immunoglobulins diluted 1:200 in TBS containing 1:25 normal human serum were added and the slides incubated for 30 minutes at room temperature. Following rinsing, ABC was incubated on the slide for a further 30 minutes at room temperature. The substrate 3,3-Diaminobenzidine (DAB) was added after washing, and after a further rinse in TBS, the slides were counterstained in Mayer's hematoxylin, dehydrated, and mounted.

Virus propagation and infectivity. CMV strain AD169 was propagated on a human embryonic lung cell line (HEL) screened for absence of bacterial, fungal, and mycoplasma contamination. Virus was harvested from the culture fluid, clarified by centrifugation at 500 × g, and stored at −70°C until use. Virus preparations were titrated in 10-fold dilutions in quadruplicate and inoculated onto bone marrow fibroblast monolayers in 96-well microtiter plates. Wells were observed after 14 days culture at 37°C in 5% CO₂ for the occurrence of cytopathic effect (CPE) and the minimum virus dose that caused CPE in 50% of the wells, the TCID₅₀, was calculated by the Kaerber method. The virus stocks used had an infectivity of 6.3 × 10⁹ TCID₅₀ U/mL on marrow fibroblasts.

CMV infection protocol. For each assay, two culture flasks of similar subconfluence were chosen. After washing, 1 mL of virus
was added to one flask, which was then incubated for 1 hour at 37°C in 5% CO₂. Virus was aspirated and replaced with McCoy's 10% FCS. A second flask was used as an uninfected control by washing and replacement with control medium. Cells were incubated for 48 to 72 hours at 37°C in 5% CO₂ before cytotoxicity assays.

**EBV infection protocol.** This was performed according to a standard technique.¹³

**Chromium release assay.** Peripheral blood mononuclear cells (PBM) were obtained by centrifugation on Lymphoprep from venepunctures of posttransplant patients and normal controls. With three exceptions, recipient samples were obtained between 3 and 8 weeks post-BMT—the period previously shown for peak MHC-unrestricted cytotoxicity against EBV-infected LCL target cells.¹⁵⁻³⁰ However, three allograft recipients were tested later, at 54, 48, and 70 weeks posttransplant, respectively. Effector cells were suspended at a concentration of 5 x 10⁵/mL in RPMI 1640 medium (Flow Labs) with 10% FCS heat-inactivated for 45 minutes at 56°C. The EBV-infected LCL was labeled by incubation with 3.7 MBq ⁵¹Cr (Amersham International PLC, Aylesbury, Bucks, UK) for 90 minutes. CMV-infected and uninfected bone marrow fibroblasts, prepared as above, were trypsinized, washed, and resuspended to 200 μL with neat FCS and labeled by incubation with 3.7 MBq ⁵¹Cr for 40 minutes at 37°C in 5% CO₂. After washing twice, target cells and effectors were set up in a standard 4-hour chromium release assay with effector:target (E/T) ratios ranging from 100:1 to 4:1.⁰⁰ Percentage specific lysis was calculated from the following formula: (Experimental release − spontaneous release/Maximum release − spontaneous release) x 100.

**Cold target competition.** This was performed using a simple modification of the chromium release assay above. Duplicate wells were set up with post-BMT PBM as effectors and radiolabeled ("hot") EBV-infected LCL target cells at an E:T ratio of 100:1. Further duplicates were set up to which were added either unlabeled ("cold") EBV-infected LCL target cells or "cold" CMV-infected bone marrow fibroblasts at "cold/hot" target ratios of 5:10 and 20:1. For each ratio of cold/hot target ratio, percentage specific lysis was calculated as above. Percentage competition was calculated using the following formula: % competition = 100 x [([% specific lysis without competitor cells − % specific lysis with competitor cells]) / (% specific lysis without competitor cells)].

**CMV antigens immunofluorescence.** Cytospin preparations of CMV-infected bone marrow fibroblasts were made immediately prior to use as target cells in the chromium release assay. Slides were fixed in acetone and examined with a panel of anti-CMV antibodies by an indirect immunofluorescence technique previously described.³¹ Antibodies used were as follows: (1) MoAb specific for the 72-Kd intermediate early antigen; (2) MoAb (p7.17) directed against the gB CMV late antigen; and (3) MoAb cocktail recognizing several epitopes of the gB CMV late antigen. All antibodies were kind gifts of Dr W. Britt (University of Alabama, Birmingham). Slides were photographed using an epifluorescent microscope. Replicate cytospin preparations of target cells were examined for mycoplasma contamination using the Hoechst dye staining method.³² None was found.

**Immunophenotyping of effector and target cell adhesion molecules.** A microplate method was used³³ with fluorescein-conjugated goat anti-mouse immunoglobulin as second layer (Southern Biotechnology, Birmingham, AL). First-layer antibodies were RFT11 (CD2), (kind gift of Professor G. Janossy, Royal Free Hospital, London, UK); anti-LFA1 (CD11a) (Immunotech, Marseilles, France); anti-LFA3 (CD58) (kind gift of Professor A. Rickinson, Birmingham, UK); and anti-ICAM1 (CD54) (Serotec, Kidlington, Oxford, UK). Final preparations were read immediately using an epifluorescent microscope and were also analyzed by flow cytometry using an EPICS 2 cell analyzer (Coulter Electronics, Luton, Beds, UK).

**MoAb blocking studies of effector and target cell adhesion molecules.** For these studies, the effector and target cell preparation was modified as follows. Post-BMT PBM effectors (5 x 10⁵) were incubated with 4 μg of MoAb to ICAM1 (CD54) for 15 minutes at 4°C, washed, and resuspended to a concentration of 5 x 10⁶/mL before use in the ⁵¹Cr release assay described above. EBV-infected LCL target cells (5 x 10⁶) were incubated with 4 μg of MoAb to LFA1 (CD11a) for 15 minutes at 4°C, washed, and resuspended to a concentration of 5 x 10⁶/mL before use in the ⁵¹Cr release assay. Four combinations were then tested: (1) untreated effectors versus untreated target cells; (2) anti-ICAM1–treated effectors versus untreated target cells; (3) untreated effectors versus anti-LFA1–treated target cells; and (4) anti-ICAM1–treated effectors versus anti-LFA1–treated target cells.

**Statistics.** Statistical analysis was performed by Student t testing of paired data.

**RESULTS**

**Purity of fibroblast adherent cell layer by immunohistochemistry.** All preparations stained strongly for Vimentin, the 57-Kd intermediate filament protein. In contrast, the mono- and macrophage marker Mac-387 was present in less than 2% of cells in the adherent layer after the first passage. Tissue-specific staining was not seen for VWF, the factor VIII-related antigen expressed on endothelial cells. This demonstrates that our culture technique selected for the growth of bone marrow fibroblasts.

**Minimal cytotoxic sensitivity of CMV and EBV target cells to MHC-unrestricted killing by normal PBM.** Normal PBM show variable but generally low cytotoxicity against MHC-mismatched CMV-infected bone marrow fibroblasts. Cytotoxicity ranged from 0.0% to 17.3% at an E:T ratio of 80:1 (mean ± SEM, 5.8 ± 2.7). Normal PBM are consistently unable to kill MHC-mismatched EBV-infected LCL target cells (mean % cytotoxicity ± SEM, 1.1 ± 0.3 at E:T ratio of 80:1) as previously demonstrated.³⁰

**Differential cytotoxic sensitivity of CMV and EBV target cells to MHC-unrestricted killing by posttransplant PBM.** After BMT, this pattern is altered. With a single exception, all BMT patients showed less than 5% specific cytotoxicity against MHC-mismatched CMV-infected marrow fibroblasts at E:T ratio of 80:1 (Fig 1). Similarly, cytotoxicity of posttransplant PBM against MHC-matched CMV-infected marrow fibroblasts was also low (mean % specific ⁵¹Cr release ± SEM, 1.45 ± 1.32 at E:T ratio of 80:1), indicating lack of MHC-restricted cytotoxicity in this system. Specific cytotoxicity against CMV-infected target cells was consistently less than 5% in all allograft recipients with and without GVHD and in the two patients who subsequently died of CMV disease. Furthermore, killing of CMV-infected target cells was also less than 5% in the three allograft recipients studied later posttransplant at 34, 48, and 70 weeks, respectively, indicating MHC-unrestricted cytotoxicity against CMV targets is not apparent to at least 70 weeks post-BMT. In contrast, substantial killing of MHC-mismatched EBV-infected LCL target cells was seen (mean cytotoxicity ± SEM, 33.3 ± 7.5 at E:T ratio of 80:1, P < .001) (Fig 1). No increase in cytotoxicity was seen.
against MHC-matched EBV-infected LCL, demonstrating no evidence of MHC-restricted cytotoxicity.34

The difference between EBV and CMV cytotoxic sensitivity post-BMT is due to disparate target structure expression. To determine whether failure to kill CMV-infected marrow fibroblasts was associated with failure to express the target structures recognized by MHC-unrestricted cytotoxic cells, unlabeled CMV-infected marrow fibroblasts or EBV-infected LCL were added in increasing numbers to 51Cr-labeled EBV-infected LCL target cells in cold target inhibition studies. In the presence of a constant number of patient effector cells, unlabeled EBV-infected LCL competed effectively with 51Cr-labeled EBV-infected LCL (Fig 2). In contrast, unlabeled CMV-infected marrow fibroblasts competed poorly against 51Cr-labeled EBV-infected LCL (Fig 2), implying that the difference between EBV and CMV cytotoxic sensitivity post-BMT is due to differences in expression of target structures.

Failure to kill was not due to inadequate viral infection. Although it is probable that MHC-unrestricted killer cells recognize cellular surface antigens that are not of viral origin,35,56 an alternative explanation for failure to kill CMV-infected target cells is that the cells express no viral antigens. However, cytospin preparations of CMV-infected marrow fibroblasts prepared immediately before use as target cells in the chromium release assay showed that the 72-Kd immediate early antigen was present on more than 90% of the cells (see Methods). Furthermore, CMV "late" antigens were expressed on more than 85% of target fibroblasts.

EBV and CMV target cells differ in their surface expression of LFA1. To determine whether differences in surface expression of adhesion molecules could explain the differential MHC-unrestricted cytotoxicity between EBV and CMV target cells, we analyzed expression of LFA1 (CD11a/18), LFA3 (CD58), ICAM1 (CD54), and CD2 on EBV and CMV target cells immediately before use in our 51Cr release assay by indirect immunofluorescence and flow cytometry (see Methods). ICAM1 and LFA3 were expressed on both target cells (Fig 3A and B), while CD2 was expressed on neither. In contrast, however, LFA1 was expressed only on EBV target cells (Fig 3A) and was absent on CMV target cells (Fig 3B). This difference provides EBV target cells with an additional binding ligand compared with CMV target cells, since posttransplant effector cells express surface ICAM1 (Fig 4).

Cytotoxicity against EBV-infected target cells is inhibited by blockade of target cell LFA1 and effector cell ICAM1. If LFA1-ICAM1 ligand interaction is indeed functionally important in determining cytotoxic sensitivity, then blockade of these ligands by MoAbs against target cell LFA1 and effector cell ICAM1 should abrogate cytotoxicity. Such an effect has been shown in T-cell-mediated antiviral cytotoxicity37 and we show here that this also occurs in MHC-unrestricted cytotoxicity against viral-infected target cells. Figure 5 shows the effect of incubating posttransplant PBM effector cells and EBV-infected target cells with MoAbs to ICAM1 and LFA1, respectively, before cytotoxicity assays as detailed in the Methods. Cytotoxicity was expressed as a percentage of control performed in the absence of blocking antibodies. Blockade of EBV-infected target cells with anti-LFA1 MoAb reduced killing by posttransplant PBM effector cells to 39% and 54% of control in each case (Fig 5). Additional blockade of posttransplant PBM effector cells with anti-ICAM1 MoAb further reduced killing to 24% and 8% of control, respectively. Interestingly, blockade of effector cells alone with anti-ICAM1 MoAb did not

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**Fig 1.** Cytotoxic sensitivity of MHC-mismatched EBV- and CMV-infected target cells to post-BMT PBM. Percentage specific chromium release is shown at three different E:T ratios (see Materials and Methods). Diagonally hatched bars represent EBV-infected LCL target cells, and solid bars represent CMV-infected marrow fibroblast target cells. Each bar shows the mean ± SEM of all observations at each E:T ratio (n = 12). ( ), EBV; ( ), CMV.

**Fig 2.** Cold target inhibition studies using post-BMT PBM. Radiolabeled ("hot") target is EBV-infected LCL to which unlabeled ("cold") EBV or CMV target cells have been added at "cold/hot" ratios of 5:10 and 20:1. Percentage competition has been calculated from percent-specific chromium release in the presence and absence of cold competitors (see Materials and Methods) and plotted at each cold/hot target ratio. Solid black line shows competition by cold EBV-infected LCL target cells, and cross-hatched and diagonally-hatched lines show competition by CMV-infected marrow fibroblasts from two patients, CMV 1 and CMV 2, respectively.
reduce cytotoxicity (Fig 5), a finding that accords with other recent studies suggesting the presence of an additional ligand for LFA1, which has been designated ICAM2.

**DISCUSSION**

After BMT, MHC-restricted cytotoxic T-cell function is poor and defense against latent viruses is largely dependent on MHC-unrestricted mechanisms. The results here indicate that the difference in incidence between CMV...
disease and EBV-related disease after MHC-identical sibling transplant BMT parallels differences in the capacity of regenerating cells post-BMT to destroy the physiological target cells for the two herpesviruses and suggest that these observations may be causally related.

Among the earliest recovering effector cells following BMT are CD16+/CD3− and CD16−/CD3+ activated killer cells demonstrating high levels of cytotoxicity to EBV-infected LCL target cells as measured in a short chromium release assay (Fig 1). Such target cells are not sensitive to killing by normal PBM (see Results). The explanation for this "activated killing" of EBV-infected target cells after autologous and allogeneic transplant is unknown. However, GVHD is not responsible for the high levels of MHC-unrestricted cytotoxicity seen in this early posttransplant period, and there is no significant difference in the magnitude of such cytotoxicity between autograft and allograft recipients. Despite uncertainty over the precise nature of the activating stimulus, the nature of the mediators of this response is now known. Direct cytotoxicity against EBV-infected LCL posttransplant appears to be predominantly mediated by the CD16+/CD3− subset, although substantial activity remains in the CD16−/CD3+ fraction.

In contrast, levels of direct cytotoxicity against MHC-matched or MHC-mismatched CMV-infected target cells are low posttransplant (Fig 1). This lack of cytotoxicity does not appear to reflect the time posttransplant at which they were tested. Thus, while most samples were assayed between 3 and 8 weeks following BMT to coincide with the period of peak MHC-unrestricted cytotoxic activity, three allograft recipients who were assayed at 34, 48, and 70 weeks posttransplant, respectively, also showed lack of cytotoxicity of MHC-matched CMV-infected targets. This is in contrast to EBV-infected targets, for which allograft recipients begin to show MHC-restricted cytotoxic responses by 6 months post-BMT. Nevertheless, our assays were performed using fresh cells, in contrast to the method of prolonged in vitro incubation with interleukin-2 and coculture with irradiated stimulator cells. As the latter system is the most sensitive assessor of MHC-restricted cytotoxicity, we cannot exclude the return of MHC-restricted cytotoxicity against CMV targets after a similar 6-month period posttransplant. However, we have shown that the failure of MHC-unrestricted cytotoxicity against CMV-infected target cells persists to at least 70 weeks post-BMT.

Discrepant reports on the role of CMV infections and activated killing posttransplant have been reported. Some investigators found a strong association, while others found no clear relationship. We have found no induction of MHC-unrestricted cytotoxicity against CMV targets in recipients with GVHD or in recipients who subsequently died of CMV disease. Although the magnitude of the cytotoxicity observed against EBV-infected targets varied among individual recipients, cytotoxicity was substantially greater against EBV- than CMV-infected target cells in all patients. Indeed, only one recipient showed greater than 5% specific cytotoxicity against MHC-mismatched or MHC-matched CMV-infected marrow fibroblasts. This patient was in first complete remission of T-ALL and was the recipient of an autograft purged in vitro with anti-CD7 antibody. He displayed specific cytotoxicity against MHC-mismatched CMV-infected marrow fibroblasts of 34% at an E:T ratio of 50:1. The explanation of this isolated case of substantial cytotoxicity against CMV-infected target cells remains unclear. Nevertheless, even in this patient, the differential cytotoxicity between the two virally infected targets was preserved, since the specific cytotoxicity against the EBV-infected target was 79%. This lack of MHC-unrestricted cytotoxicity against CMV target cells appears to conflict with an earlier report. However, in contrast to our standard 4-hour chromium release assay, this study used a long 18-hour chromium release assay in which cytotoxic cytokine release is likely to augment the effect of direct cellular cytotoxicity. Indeed, using normal PBM, MHC-unrestricted cytotoxicity against CMV-infected target cells has been demonstrated in long chromium release assays (18 to 20 hours), but not in short assays of 6 to 8 hours’ duration. We have also confirmed this observation (data not shown).

We have demonstrated that lack of MHC-unrestricted direct cytotoxicity to CMV-infected marrow fibroblasts was not due to inadequate CMV infection. Strongly positive fluorescence was seen on cytospin preparations of target cells for the 72-Kd immediate early antigen and the gB CMV late antigen (see Results). Nor is failure to kill due to the absence of HLA-DR-positive accessory cells in the effector population. Although these cells have been shown to be required for MHC-unrestricted cytotoxicity of CMV-infected fibroblasts, they are present in post-BMT PBM in normal numbers.

Instead, differences in MHC-unrestricted killing are likely to represent differences in expression of target structures involved in intercellular adhesion. A number of molecules recognized by CD16+ and CD3− MHC-unrestricted cytotoxic effectors on viral and tumor target cells have been identified. Since reciprocal inhibition between herpes simplex virus (HSV)-infected and uninfected fibroblasts has been demonstrated, it is likely that the target structure on infected cells recognized by MHC-unrestricted effectors is not encoded by virus. As we observed low levels of cytotoxicity against CMV target cells in our 4-hour chromium release assay, we were unable to assess the inhibition of CMV-infected target cells in competitive assays. Instead, we used the high levels of cytotoxicity against EBV-infected LCL to assess target structure competition by CMV-infected marrow fibroblasts. The lack of competition (Fig 2) strongly suggested that disparate expression of target molecules may explain the observed differential MHC-unrestricted sensitivity.

What is the nature of these target molecules? A number of structures have been proposed, including the transferrin receptor and HLA class I molecules. Our attention turned to the surface adhesion molecules, in particular LFA1, since absence of cell surface LFA1 has been proposed as a mechanism of escape from immunosurveillance. We have shown here that EBV-infected LCL express surface LFA1, while CMV-infected marrow fibro-
blasts do not (Fig 3A and B). Since ICAM1 is present on circulating PBM effector cells post-BMT (Fig 4), there is an additional receptor-ligand interaction between EBV target cells and effector cells that cannot occur between CMV target cells and effector cells. Our observation on the functional importance of this ligand interaction (Fig 5) parallels that of other investigators in different systems.

ICAM1/LFA1 interaction has been shown to be important in antigen-independent conjugate formation, antigen-specific cytotoxic T-lymphocyte recognition, and cytolysis of a variety of target cells, including EBV-infected LCL. This ligand pair also participates in the adhesive interaction between monocytes and T lymphocytes involved in the initiation of the immune response. ICAM1/LFA1 interactions have been shown to be important in mediating NK cell responses in normal individuals, and we now show their functional importance in MHC-unrestricted cytolysis against viral-infected target cells following BMT.

Thus, our observation that CMV-infected fibroblasts are not vulnerable to direct killing by circulating effector cells post-BMT while EBV-infected LCL are readily killed may explain both the frequency of CMV reactivation and disease and also the rarity of EBV-related disease following MHC-identical sibling BMT. However, EBV-dependent lymphoproliferative syndrome (EBV-LP) is much more common after MHC-mismatched or -matched unrelated-donor allografts, where the incidence may reach 25%. We suggest that the increased risk of EBV-LP after mismatched/unrelated-donor allografts is a consequence of the more intensive pretransplant and posttransplant immunosuppression given to these patients to reduce rejection and the risk of severe GVHD. It would be of interest to see if the MHC-unrestricted effector mechanisms we have described are impaired in these patients and are unable to kill either EBV- or CMV-infected target cells.

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