Endogenous CD8+ T Cell Expansion During Regression of Monoclonal EBV-Associated Posttransplant Lymphoproliferative Disorder


*J Immunol* 1999; 163:500-506; ;
http://www.jimmunol.org/content/163/1/500

**References**

This article cites 41 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/163/1/500.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Endogenous CD8\(^+\) T Cell Expansion During Regression of Monoclonal EBV-Associated Posttransplant Lymphoproliferative Disorder\(^1\)

Vijay P. Khatri,\(^{2,*}\) Robert A. Baiocchi,\(^{2,§}\) Ruqi Peng,\(^§\) Adam R. Oberkircher,† Jean M. Dolce,‡ Pamela M. Ward,‡ Geoffrey P. Herzig,§ and Michael A. Caligiuri\(^3§\)

There are experimental data which suggest that the primary immune effector cell responsible for maintaining immune surveillance against the outgrowth of EBV-transformed B cells in humans is the CTL, but in vivo proof of this is lacking. In this study we perform a series of cellular and molecular assays to characterize an autologous, endogenous immune response against a transplantation-associated, monoclonal, EBV\(^+\) posttransplant lymphoproliferative disorder (PTLD). Following allogeneic bone marrow transplantation, a patient developed a monoclonal PTLD of donor B cell origin. With a decrease in immune suppression, we document the emergence of endogenous, donor-derived CD3\(^+\)CD8\(^+\) CTLs, followed by regression of the PTLD. The TCR V\(\beta\) repertoire went from a polyclonal pattern prior to the development of PTLD to a restricted TCR V\(\beta\) pattern during the outgrowth and regression of PTLD. Donor-derived CD3\(^+\)CD8\(^+\) T lymphocytes displayed MHC class I-restricted cytolytic activity against the autologous EBV\(^+\) B cells ex vivo without additional in vitro sensitization. The striking temporal relationship between the endogenous expansion of a TCR V\(\beta\)-restricted, CD3\(^+\)CD8\(^+\) population of MHC class I-restricted CTL, and the regression of an autologous monoclonal PTLD, provides direct evidence in humans that endogenous CD3\(^+\)CD8\(^+\) CTLs can be responsible for effective immune surveillance against malignant transformation of EBV\(^+\) B cells. *The Journal of Immunology, 1999, 163: 500–506.

Epstein-Barr virus is a \(\gamma\)-herpesvirus that is carried as an asymptomatic and persistent infection within pharyngeal epithelium and mature B lymphocytes by \(>90\%\) of adults worldwide. While EBV-infected B lymphocytes have the potential for transformation and uncontrolled proliferation in vivo, this is thought to be normally prevented by virus-specific CTL surveillance (1–3). However, the identification of cellular components that confer this protection in vivo remains uncertain. The suppression or elimination of this protection is thought to result in the development of an EBV-associated lymphoproliferative disorder (4, 5).

EBV-associated lymphoproliferative disorder occurs with high frequency in certain congenital, acquired, and iatrogenic immunodeficient states, including the profound immunosuppression following solid organ or bone marrow transplantation (BMT). The use of T cell-depleted donor bone marrow, or the administration of intensive anti-T cell therapy in the setting of severe graft-vs-host disease (GVHD), escalates the risk of developing posttransplant lymphoproliferative disorder (PTLD) following allogeneic BMT (4). The spectrum of PTLD ranges from polyclonal B cell hyperplasia to monoclonal immunoblastic lymphoma (5, 6). Although polyclonal PTLD has been shown to regress following withdrawal of immune suppressive therapy (7), monoclonal disease demonstrates intrinsic resistance to conventional therapy and usually runs a fatal course (8). Recent success with the delivery of in vitro-generated CTLs to patients with PTLD suggests that restoration of host immunity may be the most promising therapeutic strategy in the control of these “opportunistic” malignancies (43).

The in vitro inhibition of EBV-induced B cell transformation in the presence of autologous T lymphocytes (1) and the demonstration of CTL-mediated reversal of EBV\(^+\) lymphoblastoid cell line outgrowth in xenografted SCID mice (3) also strongly support the notion that T cells are critical in the control of EBV infection. Likewise, the clinical observation that PTLD regresses following relaxation of immunosuppressive therapy is consistent with this. However, as recently reviewed by Rickinson et al. (11), direct evidence that endogenous EBV-specific CTLs can mediate regression of PTLD in these patients is still lacking.

In the current report, we provide a detailed characterization of the emergence of a donor-derived, monoclonal PTLD following allogeneic BMT and document its regression following the endogenous expansion of donor-derived CD3\(^+\)CD8\(^+\) CTL in blood.

Materials and Methods

Case report

A 31-yr-old Japanese man was diagnosed with Philadelphia chromosome positive, t(9;22), chronic myelogenous leukemia. He underwent allogeneic BMT with a conditioning regimen that consisted of etoposide (1.8 g/m\(^2\) over 2 days), cyclophosphamide (60 mg/kg/day for 3 days), and total body irradiation (10 G in five fractions). He received an infusion of bone marrow (2.62 \(\times\) 10\(^8\) cells/kg) from an HLA-matched unrelated donor (donor and recipient HLA typing: A2, B46, DR8, DQ1, DQB1-06, DQB1-08) that was not T cell depleted. This treatment was performed after obtaining informed consent.
consent, approved by the Institutional Review Board at Roswell Park Cancer Institute.

Prophylaxis against GVHD was initiated 48 h prior to bone marrow infusion (i.e., day 2) with cyclosporine A (CsA, 5 mg/kg/day). One dose of methotrexate (10 mg/m²) was administered on day 1. Solumedrol was initiated on day 1 at a dose of 1 mg/kg/day and was increased to 9 mg/kg/day on day 21 following the development of skin GVHD. Colonoscopy was performed on day 29 to evaluate high volume diarrhea and showed GVHD. OKT3 mAb was therefore administered at 1 mg/kg/day. Severe GVHD persisted, so two consecutive 8-day courses of 2-chloro-deoxyadenosine (2-CDA; 0.02 mg/kg/day) were administered. By day 56 the severity of the GVHD had decreased.

On day 62 flow cytometric analysis of blood showed a phenotype consistent with B cell excess (CD19⁺CD56⁻; CD3⁺CD20⁺CD3⁻) and T cells (CD3⁺CD8⁺) in the blood of the patient throughout the clinical course. Relevant therapeutic events occurring during the clinical course are indicated below the horizontal axis. B, Temporal course of absolute number of lymphocyte subsets (CD19⁺CD20⁺CD3⁻; CD3⁺CD8⁺; or total CD3⁺) in peripheral blood (cells/mm³). C, Histograms of lymphocyte subsets on day +62 show that 86% of gated lymphocytes are CD19⁺, whereas 7% express CD3 (left panel). B lymphocytes are predominantly CD20⁺CD23⁻, consistent with a B lymphoblast phenotype (middle panel). Ninety-six percent of CD19⁺ B lymphocytes express surface Ig with ALC (right).

Flow cytometric and Southern blot analyses

Two-color phenotypic analysis of blood using directly conjugated mAbs was performed as described (12) using a lymphocyte gate. Routine methods for DNA isolation, digestion, electrophoresis, Southern blotting, and 32P radiolabeling of probes were followed (9). DNA probes for the JH region of the IgH gene (10), for EBV DNA (13), and for the constant region of the TCR β-chain gene (14) have been described.

Identification of B cell and T cell gene rearrangement by PCR

To identify the specific IgH V-D-J gene rearrangement associated with the B cell lymphocytosis, DNA was isolated from PBMC on day +75. The sequences of the seven IgH-V family-specific 5' primers and the four IgH-J generic 3' primers, along with PCR amplification conditions, have been described (15). Following amplification, one distinct band appeared which was excised and sequenced. DNA from day +65 and day +68 PBMC were also amplified and sequenced; they were found to be identical. Subsequently, IgH-V5 (5'-TGGCGCCAGATGCCGGGAAAG-3') and IgH-J4 (5'-GAGGAGACGGTGACCAGGGTTCCCTGG-3') primers were used to detect this IgH gene rearrangement in DNA extracted from serial blood and tissue samples.

Analysis of the TCR β gene repertoire was undertaken at indicated time points and from indicated tissues. RNA was isolated using RNAzol (Tel-Test, Freindwood, TX) and reversed transcribed into first-strand cDNA following the manufacturer’s recommendations (Clontech, Palo Alto, CA)
TCR primers, each paired with a single Cb-specific cell lysis as described previously (17).

Identification of EBV latent membrane protein-1 (LMP-1)

RNA isolation and reverse transcription (RT) were performed as above. Thirty-five cycles of PCR amplification and reverse transcription were performed with 25 different TCR Vβ 5' primers, each paired with a single Cβ-specific 3' primer as directed (Clontech). An identical aliquot of the cDNA was used for amplification of the TCRα gene constant region using C 5'α and C 3'α primers as an internal control to verify the integrity of the cDNA (data not shown). Water was used as negative control.

Microsatellite PCR analysis

The details of performing PCR amplification of the microsatellite locus 220yh4 for purposes of distinguishing donor versus recipient cells have been described (16).

DNA sequencing

PCR products were first purified using the Quick PCR purification kit (Qiagen, Chatsworth, CA) and sequenced on an Applied Biosystems model 373 Stretch DNA sequencing system (Perkin-Elmer). Sequences were analyzed using GenBank databases (IntelliGenetics, Campbell, CA).

Cytotoxicity assays

Target PBMC (day +62) and effector PBMC (day +113) were collected from the patient and immediately frozen viably without further sensitization in vivo. On the evening prior to the cytotoxicity assay, cells were thawed, washed, resuspended in RPMI 1640 medium supplemented with 10% FCS, and cultured separately overnight for equilibration. A total of 50 pM of IL-2 (10 U/ml) was added to day +113 EBV-LMP-1 mRNA (5'-CCTCCGGACCCCTCAACAAG-3'; 5'-GAGATGATGACGC CCCCCA-3'). β-Actin was amplified from cDNA samples with commercially available primers (Perkin-Elmer, Norwalk, CT).

Results

Characterization of donor-derived PTLD and expansion of CTL in vivo

The temporal course of the B cell lymphoproliferation and the endogenous, autologous cellular immune response that followed were assessed with serial flow cytometric analyses over a 3-mo period (Fig. 1, A and B). Sixty-two days following BMT, the first changes indicating a clonal B cell expansion were noted, with 86.7% of lymphocytes expressing CD19 (Fig. 1C). Ninety-six percent of CD19 B lymphocytes expressed surface Ig with a light chain (ALC), indicating a clonal population of B lymphocytes. This same clonal population expressed CD20 and CD23, consistent with a B cell lymphoblastic phenotype commonly associated with EBV transformation (Fig. 1C) (18). The monoclonal population of B lymphocytes was confirmed by Southern blot analysis and also was shown to contain the EBV genome (Fig. 2, A and B). PCR amplification of microsatellite DNA isolated from FACSorted subsets of lymphocytes from day +62 showed the monoclonal B cell population was of donor origin (Fig. 2C). RT-PCR analysis of RNA extracted from blood drawn the same day revealed an EBV-LMP-1 gene product that was present but somewhat smaller than the B95.8 EBV strain LMP-1. Sequence analysis demonstrated numerous point mutations and deletions between the coordinates 168,611 and 168,225, virtually identical to the LMP-1 transcript variant described in the C15 nasopharyngeal carcinoma isolate (data not shown) (19).

With documentation of monoclonal, donor-derived EBV PTLD, IFN-α, ACV, and IVIg were administered without effect.

2-CDA, administered earlier as an immunosuppressive agent, has no known activity against EBV-associated lymphoma (20). On day +81, 18 days after the reduction of immunosuppressive therapy, an expansion of CD3 CD8+ T cells was noted, with a concomitant decrease in CD19+ B cells (Figs. 1A and 3A). The CD4:CD8 ratio was 1:13, and nearly all T cells showed an activated phenotype with HLA-DR expression (Fig. 3A). Without further pharmacologic or immunologic manipulations, by day +97 the CD3+CD8+ T cell population was some 44% of peripheral blood lymphocytes and the clonal CD19+ population was reduced to 14% (Fig. 1A). Absolute numbers of CD19+ and CD3+ lymphocytes began to decline in parallel at this time (Fig. 1B). Blood from day +97 was analyzed for TCR gene rearrangement by Southern blot analysis and showed no evidence of clonal rearrangement (data not shown). Microsatellite analysis of DNA isolated from sorted T lymphocytes showed complete donor origin of T lymphocytes.
RT-PCR for the detection of the LMP-1 transcript was 1 cell in 10^6 sensitivity of detecting 1 cell in 10^5, while the sensitivity of the obtained from the same tissues and assayed for the EBV-LMP-1 and lymph node tissues obtained at autopsy. In addition, RNA was BMT, along with serial post-BMT blood samples, were assessed segment. Donor and recipient bone marrow obtained prior to ically associated with the monoclonal PTLD. Sequencing demon-

FIGURE 3. A. Histograms of lymphocyte subsets on day +81. CD19+ B lymphocytes comprise 41% of gated lymphocytes, 88% of which demonstrate a ALC restriction (left panel). T cells are predominantly CD3+CD8+ (52%, middle panel) and express HLA-DR. B. Histograms of lymphocyte subsets on day +103. The persistence of CD3+CD8+ CTLs is seen despite a marked reduction in the CD7+CD19+ B cell population. The majority are HLA-DR+. C. Histograms of lymphocyte subsets from day 113 show 1.5% of gated lymphocytes to express CD19 (left panel), with the persistence of a distinct population of CD3+CD8+ lymphocytes (middle panel); 68% of gated lymphocytes express HLA-DR (right panel).

Analysis of TCR Vβ gene repertoire in response to PTLD
We next characterized the TCR Vβ gene repertoire in blood over time. As all cells following BMT were of donor origin (Figs. 2, C and D), the TCR Vβ profile of the donor T cells before BMT were also analyzed. This profile showed a typical polyclonal pattern (Fig. 5A). At day 64, 2 days following the detection of a clonal population of B lymphoblasts, the TCR Vβ repertoire was unchanged with the exception that TCR Vβ1 became undetectable and Vβ3 and -23 became apparent for the first time (Fig. 5B). Twenty-three days after the detection of circulating clonal EBV+B cells (i.e., day +83), the TCR Vβ repertoire was unchanged with the exception of Vβ1 again being detected (Fig. 5C). By day +97, PTLD was diminishing (Fig. 1B), LMP-1 transcript became undetectable in blood (Fig. 4B), and the percent CD3+CD8+ CTL were at their peak (Fig. 1A). At this time there was a switch from a polyclonal to a restricted pattern of TCR Vβ usage, with a predominance of Vβ1, -2, -3, -4, -6, -8, -9, and -23 in blood (Fig. 5D).

As the liver was the site of fatal hepatic GVHD but showed no molecular evidence of B cell tumor, we next determined if the TCR Vβ repertoire of T lymphocytes present in the liver at the time of death was similar to that observed in blood when the EBV LMP-1 transcript was undetectable. The T cells in the liver expressed TCR Vβ3, -4, -6, and -8, all of which were present in the blood during the regression of the PTLD, as well as TCR Vβ7, -22, and -24 that were not in the earlier blood sample (Fig. 5E).

Cytotoxic activity of ex vivo CTL against EBV+B lymphoblasts
To determine if the CD3+CD8+HLA-DR+ CTL present in blood during the resolution of PTLD were indeed MHC class I-restricted cytolytic effectors, a standard ⁵¹Cr-release cytotoxicity assay was performed using PBMC from day +62 (86% CD19, CD20, CD23, surface Ig λLC, and EBV+) as targets (T) and PBMC from day +113 (66% CD3+CD8+HLA-DR+) as effectors (E). All cells
were donor derived (Fig. 2, C and D). No additional in vitro sensitization was performed prior to the 4-h assay. Cytotoxicity at an E:T of 20:1 in the presence of isotype control Abs was 76.3 ± 4.2%. In the presence of Abs reactive against MHC class I and CD8, cytotoxicity was 63 ± 1.3% (Fig. 6). Thus, donor-derived CTL that expanded in peripheral blood during the simultaneous reduction of donor-derived CD19+, monoclonal, EBV+ B lymphoblasts displayed MHC class I-restricted cytotoxicity against this population.

Discussion

This report provides what we believe to be the first detailed cellular and molecular characterization of an endogenous autologous immune response to monoclonal PTLD. We document the progressive clonal expansion of a donor-derived EBV+ B lymphoblast population expressing an LMP-1 transcript variant, which grew to represent >85% of circulating lymphocytes. With a decrease in iatrogenic immunosuppressive therapy, there was a gradual decline in this monoclonal EBV+ B cell population and a progressive, endogenous expansion of donor-derived T cells. The vast majority of these T cells had a CD3+CD8+HLA-DR+ phenotype and restriction of the TCR Vb repertoire. The expression of HLA-DR is consistent with an activated CTL effector population (23). TCR Vb repertoire restriction has been well documented following Ag-specific (24) and allospecific (20) responses and after allogeneic BMT with GVHD (25, 26). A similar restriction of the TCR Vb occurs when generating EBV-specific CTL in vitro (27, 28), in vivo following primary infection with EBV (29, 30), and in healthy adults who are seropositive for EBV (31). The relatively sudden appearance of the TCR Vb repertoire restriction, the predominance of the CD8+HLA-DR T cell phenotype, and the MHC-restricted cytotoxic activity demonstrated against the autologous EBV+ tumor cells ex vivo are all consistent with an autologous, Ag-specific T cell response. The possibility of this T cell expansion representing an allospecific response against recipient tissues cannot be fully excluded. However, the parallel decline of CTL with the decrease in viral load (Fig. 1B) despite the persistence of severe clinical GVHD until death would, in addition to the other data, also argue against this. The nearly complete abrogation of cytotoxic activity in the presence of anti-MHC class I mAb is consistent with earlier reports showing the same inhibition of CTL-mediated lysis of EBV+ LCLs in vitro (32), and argues against NK cells as effectors. This is because NK cell cytotoxic activity against autologous EBV+ LCLs increases in the presence of MHC class I blockade (33).

The Vb-restricted population of CTL persisted while the circulating B cell population was reduced to <7% of lymphocytes, and the LMP-1 mRNA transcript became impossible to detect in blood, spleen, liver, or lymph node despite a highly sensitive RT-PCR.
GVHD, known to be caused by alloreactive T cells (41). The TCR
ination of both the EBV-LMP-1 transcript and the B cell clone
of latent gene usage by EBV in maintenance of long-term persis-
tained at autopsy support recent findings that describe the plasticity
described in vivo (34, 37– 40). Thus, DNA PCR evidence docu-
the patient in this study was likely to have maintained a nonim-
showed evidence of the B cell clone by DNA PCR suggests that
anted (36). Thus, the absence of LMP-1 transcript in tissues that
munologically recovering bone marrow transplant recipient,
patient in this study (35). In an immunocompetent host or an im-
assay. LMP-1 transcripts are believed to be expressed only in pro-
ferating EBV \( ^{+} \) B cell immunoblasts that are highly immunogenic
and accordingly sensitive to EBV-specific CTL recognition in vivo
(34). Furthermore, the LMP-1 gene product has been shown to
possess immunodominant epitopes utilized by EBV-specific CD3 \(^{+}\) CD8 \(^{+}\) T lymphocytes in HLA A2 individuals, similar to the
patient in this study (35). In an immunocompetent host or an im-
munologically recovering bone marrow transplant recipient,
LMP-1 expressing B cell immunoblasts should be effectively erad-
icated (36). Thus, the absence of LMP-1 transcript in tissues that
showed evidence of the B cell clone by DNA PCR suggests that
the patient in this study was likely to have maintained a nonim-
munogenic, clonal population of EBV \( ^{+} \) B cells expressing a latent
gene profile restricted to EBV-encoded nuclear Ag 1 (EBNA1) and
LMP-2A (latency type 1) following the T cell response. EBV has
been shown to establish long-term infection and latency type 1
gene expression in mature, resting memory B lymphocytes of nor-
mal, asymptomatic carriers (37). Indeed, cellular immune-mediat-
ed mechanisms resulting in the transition of activated, prolifer-
ating EBV \( ^{+} \) B lymphoblasts to a quiescent, resting state have been
described in vivo (34, 37–40). Thus, DNA PCR evidence docu-
menting the presence of the B lymphocyte clone in tissues ob-
tained at autopsy support recent findings that describe the plasticity
of latent gene usage by EBV in maintenance of long-term persis-
tence within the memory B cell compartment.
The liver was the only organ examined that demonstrated eli-
mination of both the EBV-LMP-1 transcript and the B cell clone
transformed by the virus. The liver was also the site of fatal
GVHD, known to be caused by alloreactive T cells (41). The TCR
\( \beta \) repertoire detected within the liver parenchyma at the time of
fatal GVHD showed some overlap with that which predominated
during the CTL expansion in vivo and which demonstrated MHC
class I-restricted cytotoxic activity against EBV \( ^{+} \) B cell lympho-
blasts in vitro. Thus, it is possible that the donor CTL responsible
for the elimination of autologous PTLD could have also contrib-
uted to the fatal hepatic GVHD that occurred in the allogeneic
issues. However, we were unable to provide any proof of this in
this analysis. If true, it would support earlier in vitro studies dem-
onstrating dual specificity of CTL for autologous EBV-transformed
 cell lines or a single EBV epitope and HLA alloantigens (42). Further, it would underscore the importance of eliminating
alloreactive CTL from autologous EBV-specific CTL generated ex vivo prior to their infusions in vivo for the treatment of PTLD (43).

Several aspects of this report provide new information with re-
gard to the in vivo control of PTLD. We provide direct evidence
that withdrawal of immune suppression in a patient with GVHD
following allogeneic BMT resulted in the emergence of endoge-
nous donor-derived CD3 \(^{+}\) CD8 \(^{+}\) HLA-DR \(^{+}\) T cells that demon-
strated MHC class I-restricted cytolytic activity against the mono-
clonal donor-derived B cell PTLD ex vivo. The endogenous emergence of this oligoclonal population of CTL was associated
with the simultaneous regression of the monoclonal PTLD in vivo.
This report therefore provides additional evidence in support of the
generally accepted notion that autologous EBV-specific CTL are
responsible for the normal immune surveillance against PTLD.

Acknowledgments

We thank Drs. John Yates (Roswell Park Cancer Institute, Buffalo, NY),
Jeffrey Cohen (National Institutes of Health, Bethesda, MD), and Peter
Doherty (St. Jude Children’s Research Hospital, Memphis, TN) for helpful
discussions; Pamela Evans and Eileen Healy for tissue procurement;
and Wendy Ralph for secretarial assistance. We thank Mary-Beth Dell and
Gaia Barresi for additional technical assistance and Todd Fehninger for
assistance with flow cytometric analysis.

References

1. Rickinson, A. B., and D. J. Moss. 1997. Human cytotoxic T lymphocyte re-
sponses to Epstein-Barr virus infection. Annu. Rev. Immunol. 15:403.
the development of Epstein-Barr virus (EBV)-associated lymphoproliferative disease in
scid/scid mice reconstituted i.p. with EBV-responsive human peripheral
and R. J. O’Reilly. 1996. Human Epstein-Barr virus (EBV)-specific cytotoxic T
lymphocytes home preferentially to and induce selective regressions of autolo-
gous EBV-induced B cell lymphoproliferations in xenografted CB-17 scid/scid
mice. J. Exp. Med. 183:1215.
Epstein-Barr virus associated lymphoproliferative disease. Lab. Invest. 67:5.
and R. E. Michler. 1995. Correlative morphologic and molecular genetic analysis
demonstrates three distinct categories of posttransplantation lymphoproliferative
lymphoproliferative diseases: implications for understanding their pathogenesis
7. kvinn, G. T., C. K. Porter, M. H. Sawa, S. Iwatsuki, B. P. Griffith,
versibility of lymphomas and lymphoproliferative lesions developing under cy-
Structure of the human immunoglobulin mu locus: characterization of embryonic
from the Epstein-Barr viral genome that permits stable replication of recombinant
O. L. Hanson, and J. Love. 1991. Gene rearrangements in the diagnosis of lymph-
15. Greenberg, S. J., Y. Choi, M. Ballow, T. L. Du, P. M. Ward, M. H. Rickert,
S. Frankel, S. H. Bernstein, and M. L. Brecher. 1995. Profile of immunoglobulin
heavy chain variable gene repertoires and highly selective detection of malignant
1995. Description of an efficient and highly informative method for the evaluation

FIGURE 6. Autologous MHC class I-restricted cytotoxic activity. PBMC from day +113 (66% CD3 \(^{+}\) CD8 \(^{+}\) T cells, effectors) were plated against \( ^{31} \)Cr-labeled PBMC obtained on day +62 (76% EBV \(^{+} \) B lympho-
blasts, targets). The assay was performed at an E:T ratio of 20:1 in the
presence of nonreactive isotype control mAbs or anti-HLA class I and
anti-CD8 mAbs. Results represent the mean ± SE of triplicate wells.

Percent Cytotoxicity

Day 62 PBMC + isotype mAb

Day 62 PBMC + Anti HLA A,B,C

40 80

0 20

Target Population

0 100

0 20 40 60 80 100

Day 62 PBMC + Anti HLA A,B,C + Anti CD8

Day 62 PBMC + Anti HLA A,B,C + Anti CD8
of hematopoietic chimerism following allogeneic bone marrow transplantation. Bone Marrow Transplant. 16:695.


