Ganciclovir Treatment of Herpes Simplex Thymidine Kinase-Transduced Primary T Lymphocytes: An Approach for Specific In Vivo Donor T-Cell Depletion After Bone Marrow Transplantation?

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Allogeneic bone marrow transplantation (BMT) is associated with a severe complication—graft-versus-host disease (GVHD). Although effectively preventing GVHD, ex vivo T-lymphocyte marrow depletion unfortunately increases graft rejection and reduces the graft-versus-leukemia (GVL) effect. The ex vivo transfer of the herpes simplex thymidine kinase (HS-tk) suicide gene into T cells before their infusion with hematopoietic stem cells could allow for selective in vivo depletion of these T cells with ganciclovir (GCV) if subsequent GVHD was to occur. Thus, one could preserve the beneficial effects of the T cells on engraftment and tumor control in patients not experiencing severe GVHD. To obtain T cells specifically depleted by GCV, we transduced primary T cells with a retroviral vector containing the HS-tk and neo-resistance (NeoR) genes. Gene transfer was performed by coculturing PHA ± CD3- or alloantigen-stimulated purified T cells on an irradiated retroviral vector producer cell line or by incubating the T cells in supernatant from the producer. Subsequent culture in G418 for 1 week allowed for the selection of transduced cells. GCV treatment of interleukin-2–responding transduced and selected cells resulted in greater than 80% growth inhibition, whereas GCV treatment of control cells had no effect. Similarly, the alloreactive reactivity of HS-tk-transduced cells was specifically inhibited by GCV. Combining transduced and nontransduced T cells did not show a bystander effect, thus implying that all of the cells inhibited by GCV were indeed transduced. Lastly, studies involving the transduction of the HUT-78 (T-lymphoma) cell line suggest that stable expression of HS-tk can be maintained over 3 months in vitro in the absence of G418. In summary, we have established the feasibility of generating HS-tk–transduced T cells for subsequent in vivo transfer with hematopoietic stem cells and, if GVHD occurs, specific in vivo GCV-induced T-cell depletion in allogeneic BMT recipients.

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approach should allow for the dissection of post-BMT alloimmunological activity and, therefore, considerably enhance our comprehension of the mechanisms involved in GVHD, GVL, and graft rejection.

Several issues need to be addressed when investigating means of obtaining GCV-sensitive donor primary T cells: (1) because the target cells are primary T cells, the HS-tk transfer and subsequent selection process should be performed within a relatively short time-frame; (2) in contrast to other gene therapy studies,18-20 the selection process after HS-tk transfer in the allogeneic T cells must reproducibly result in a very high percentage of HS-tk−transduced GCV-sensitive cells; (3) the transduced T cells alloreactivity should be abrogated by GCV; and (4) the donor T cells should be capable of being ablated by GCV treatment for several months after BMT. Thus, long-term stable expression of the HS-tk gene in the absence of G418 is essential.

In the present study, we describe our efforts to address these various issues. We demonstrate that one can indeed generate HS-tk−transduced T cells and that use of these T cells does indeed allow for specific GCV-mediated immunomodulation.

MATERIALS AND METHODS

Cells and retroviral-containing supernatants. Peripheral blood cells were provided from healthy volunteer donors. After gradient separation (LSM; Organon Teknika, Durham, NC), the peripheral mononuclear cells were cryopreserved in 10% (dimethyl sulfoxide DMSO; Sigma, St Louis, MO) or were further enriched for T lymphocytes by counterflow elutriation separation (J-6ME centrifuge; Beckman, Fullerton, CA). The resulting cell populations were greater than 90% CD3+ T lymphocytes (data not shown). HUT-78, a CD3+ CD4+ T-cell lymphoma cell line, was kindly provided by Dr Ali Gazdar (National Cancer Institute, Bethesda, MD).

A modified LNL6 retroviral vector (G1TK1SvNa), provided by Genetic Therapy, Inc (Gaithersburg, MD), was used in this study. G1TK1SvNa contains an HS-tk gene under control of the retrovirus LTR and a neomycin resistance (NeoR) gene under the control of an internal SV40 promoter inserted 3' to the HS-tk gene. The vector-producing 3T3 fibroblast cell line (PA317/G1TkVNa) produced 1 x 105 colony-forming units (CFU)/mL and was free of replication-competent virus. A limited number of experiments were performed with a similar vector (G1TK1SvNa) with identical HS-tk andNeoR sequences and promoters and modified flanking sequences resulting in a higher viral titer (2 x 105 CFU/mL).

Transduction and selection procedure. Peripheral blood mononuclear cells or purified T lymphocytes in complete medium (CM) containing RPMI 1640 (GIBCO, Grand Island, NY), 15% human AB serum (ABI, Columbia, MD), penicillin/streptomycin, L-glutamine, and HEPES (Whittaker Bioproducts, Walkersville, MD) were stimulated with (1) phytohemagglutinin (PHA; 1 μg/mL; Burroughs-Wellcome, Raleigh, NC), with (2) irradiated allogeneic mononuclear cells and recombinant human interleukin-2 (IL-2; 500 U/mL); (kindly provided by Hoffmann-La Roche, Nutley, NJ), or with (3) a combination of CD3 monoclonal antibody (MoAb; CD3-coated flasks; Applied Immune Systems, Menlo Park, CA), IL-2, and PHA and incubated at 37°C and 10% CO2. After 3 to 5 days in culture, gene transfer was performed by coculturing the target cells on the irradiated (2,500 R) retroviral vector producer cells for 2 to 3 days or incubating the cells in the supernatant from the producer for 4 hours. Polybrene (4 μg/mL; Sigma) as well as IL-2 (500 U/mL) were added during the transduction procedure. One to two days later, transduced cells, were selected for by incubation for 3 to 7 days in CM with G418 (300 to 900 μg/mL; Geneticin; GIBCO) and IL-2 (500 to 1,000 U/mL). HUT-78 cells were similarly transduced followed by selection over a 3-week period in G418 at concentrations up to 1,400 μg/mL.

Proliferation assay. Transduced G418-selected cells were washed and cultured for 5 days in 96-well round-bottom plates (103 cells/well), with or without various doses of GCV (0.1 to 10 μg/mL; Syntex, Palo Alto, CA) and (1) IL-2 (500 U/mL) or (2) irradiated (4,000 R) allogeneic mononuclear cells (103 cells/well) and IL-2 (50 U/mL). Radiolabeled thymidine (H'TdR; 1 μCi/well; specific activity, 6.7 Ci/mmol; Dupont Co, Boston, MA) was added for the last 12 to 18 hours of culture before harvesting the DNA and counting in a beta scintillation counter (Wallac 1410; Wallac, Gaithersburg, MD). The results are expressed as counts per minute (CPM) and are presented as means of triplicate cultures ± standard deviation. The effects of GCV on cell growth are expressed as a percentage of growth inhibition.

Polymerase chain reaction (PCR) analysis. The PCR was used to screen for the presence of NeoR gene in the T lymphocytes after G1TK1SvNa retroviral transduction. Genomic DNA from T lymphocytes as well as from G1TK1SvNa/PA317 cells were prepared by standard proteinase K-sodium dodecyl sulfate (SDS) treatment and phenol-chloroform extractions. Each 100-μL reaction included 1 μg of genomic DNA using standard reaction conditions: 1.5 mmol/L MgCl2, 0.2 mmol/L of each dNTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, 50 mmol synthetic primers (left-hand primer: 5'-GGTTGAGAGGCTATTCGGCTATGA-3'; and right-hand primer: 5'-TCTCTGACAGACGCGCTTCC-3'), and 0.01 U/μL of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Emeryville, CA). DNA was amplified using 30 cycles of incubation at 94°C (30 seconds), 61°C (30 seconds), and 72°C (45 seconds), followed by a 10-minute extension period at 72°C. The amplified fragments (10 μL) were separated by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining. The size was determined using DNA molecular weight markers (φ X 174; Boehringer Mannheim, Neylan, France). Beta-actine DNA was amplified (left-hand primer: 5'-ATCATGT'TTGAGACCTTCAA-3' and right-hand primer: 5'-CATCCTTTGCTCGAAGTCCA-3') with the same protocol and used as an internal control for DNA integrity and loading.

DNA mixtures prepared from the viral producer line and control peripheral blood lymphocytes (100% to 0%, 60% to 40%, 50% to 70%, 70% to 90%, and 5% to 95%) were similarly transduced and incubated for 3 days in the presence of GCV at doses of both 0.1 and 1 μg/mL (67% and 87% growth inhibition, respectively; Fig 1). Initial addition of an CD3 MoAb or an allo-Ag to stimulate the T cells gave similar results (data not shown). Both concentrations of GCV had little or no effects on control nontransduced cells and are concentrations achieved in vivo during GCV

RESULTS

The transfer of the HS-tk/NeoR genes into primary T cells, followed by G418 selection, allows for specific GCV-induced growth inhibition. Coculture of PHA-stimulated T lymphocytes on the irradiated retrovirus-producing packaging cell line or incubation of the stimulated T cells in the supernatant from the producer, followed by 7 days of selection in G418, resulted in T cells with IL-2−-driven proliferation specifically inhibited by GCV at doses of both 0.1 and 1 μg/mL (67% and 87% growth inhibition, respectively; Fig 1). Initial addition of an CD3 MoAb or an allo-Ag to stimulate the T cells gave similar results (data not shown). Both concentrations of GCV had little or no effects on control nontransduced cells and are concentrations achieved in vivo during GCV

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treatment for cytomegalovirus infection. When used at a higher concentration (10 μg/mL), GCV treatment also inhibited the growth of nontransduced cells with, however, a persistent difference between transduced-selected cells and control cells (80% to 90% growth inhibition vs 40% to 60%).

In the experiment described in Fig 1, selection of transduced cells in 300 or 600 μg/mL of G418 resulted in cell populations that were inhibited to the same extent by GCV treatment. This finding was variable from donor to donor. Selection in shorter duration (3 days) of G418 selection was associated with reduced GCV-induced growth inhibition (data not shown). However, 1 week of selection at G418 concentrations greater than 500 μg/mL always resulted in cells inhibited by GCV. In the absence of G418 selection, posttransduction T cells were not inhibited by GCV, suggesting that before G418 selection, the percentage of cells expressing the HS-tk/NeoR genes was very low.

Of interest, although viable control (nontransduced) cells persisted after 1 week of G418 (300 and 600 μg/mL) selection, these cells were not capable of subsequently responding to IL-2 (Fig 2), suggesting that, although G418 does not kill all the nontransduced cells by 7 days, the remaining ones have lost their proliferative potential. On the other hand, HS-tk/NeoR gene-transduced and G418-selected cells retain the capability of responding to IL-2 (Fig 2 and Table 1).

Gradient separation and/or extended culture after G418 selection results in cells with an enhanced proliferative response to IL-2 and maintained GCV-induced growth inhibition. The reduced proliferative response to IL-2 of the transduced and G418-selected cells suggested that, among the "live" cells plated with IL-2, only a fraction of the cells were indeed "resistant" to G418 and had retained full proliferative capabilities. This hypothesis was confirmed by the finding that a gradient separation (LSM) after G418 selection (600 μg/mL) or a further 6-day culture in the absence of G418 resulted, after a significant loss of cells, in T cells with an enhanced proliferative response to IL-2 (Table 1). These findings suggest that the additional separation procedure or culture period before assessing GCV-induced growth inhibition resulted in the removal of the remaining G418-sensitive cells. Consistent with this hypothesis, gradient separation of control (nontransduced) cells still alive after G418 selection resulted in very few cells unable to respond to IL-2. Most importantly, the gradient separation and/or further culture did not alter the sensitivity of the T cells to GCV with GCV-induced growth inhibition greater than 80% (Table 1).

A semiquantitative genomic analysis of the transduced and G418 selected T cells by PCR confirmed the presence of the transgene in a high proportion of the cells after G418 selection (Fig 3). Interestingly, the proportion of provirally marked T cells was higher after an additional week of culture after G418 selection then right after G418 selection. This finding is consistent with our functional data and confirms that, after 1 week of G418 selection, a proportion of the surviving cells are nontransduced and that most of these remaining nontransduced cells will not survive an additional week of culture.

Prolonged culture of Tk-expressing T cells in GCV results in significant cell loss as well as IL-2 responsiveness. Transduced and G418-selected cells as well as control cells were
Table 1. Effects of Density Gradient Separation and/or 6-Day Culture on the IL-2 Responsiveness and GCV-Induced Growth Inhibition of HS-tk-Transduced (G1TkSvNa) Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>G418 (μg/mL)</th>
<th>Day 14</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No GCV</td>
<td>GCV</td>
<td>No GCV</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>185.8 ± 15.7</td>
<td>245.1 ± 12.8 (0)</td>
</tr>
<tr>
<td>Control</td>
<td>600</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>TK</td>
<td>0</td>
<td>178.8 ± 64.4</td>
<td>174.7 ± 31.5 (0)</td>
</tr>
<tr>
<td>TK</td>
<td>300</td>
<td>7.2 ± 1</td>
<td>1.7 ± 0.3 (75)</td>
</tr>
<tr>
<td>TK</td>
<td>600</td>
<td>4.2 ± 0.3</td>
<td>0.4 ± 0.1 (90)</td>
</tr>
<tr>
<td>TK-LSM</td>
<td>0</td>
<td>330.0 ± 12</td>
<td>317.7 ± 22.7 (4)</td>
</tr>
<tr>
<td>TK-LSM</td>
<td>300</td>
<td>30.6 ± 0.9</td>
<td>6.0 ± 0.7 (80)</td>
</tr>
<tr>
<td>TK-LSM</td>
<td>600</td>
<td>23.6 ± 1.7</td>
<td>1.8 ± 0.1 (92)</td>
</tr>
</tbody>
</table>

After gene transfer and G418 selection, the T lymphocytes (TK cells) underwent on day 14 a density gradient separation (LSM) (TK-LSM cells) and/or additional 6-day culture in IL-2 without G418 before being plated with IL-2 (500 U/mL) with or without GCV (1 μg/mL). Percentages are in parentheses.

Abbreviation: NE, not evaluable because of the absence of surviving control cells.

cultured with GCV (1 μg/mL) plus IL-2 for 14 days. Cell numbers and IL-2–induced proliferative responses were measured at day 7 and 14 (Fig 4A and B). GCV treatment resulted in a progressive and selective reduction in cell numbers as well as IL-2 responsiveness of Tk-expressing cells. By day 14, there was a sevenfold decrease in cells numbers and 2 log decrease in proliferative response to IL-2 of GCV-treated transduced cells when compared with GCV-treated control cells. Because cells were adjusted and identical numbers of transduced and control T cells were plated before evaluating their proliferative response to IL-2, one can conclude that continuous exposure to GCV not only considerably reduced the number of viable transduced cells but also drastically inhibited the proliferative capabilities of the remaining cells.

GCV-induced growth inhibition of primary T cells is not mediated through a bystander effect. An HS-tk–mediated

Fig 3. PCR analysis. Ethidium bromide-stained gel of amplification products obtained using primers specific for NeoR. Products were amplified from 1 μg of mixtures of genomic DNA isolated from the producer cell line (G1TkSvNa/PA317) and control peripheral blood lymphocytes or genomic DNA isolated from transduced T cells after 1 week of G418 (A), from transduced T cells after 1 week of additional culture out of G418 (B), or from nontransduced, nonselected T cells (C).

Fig 4. Effects of continuous exposure of GCV on cell numbers and IL-2 responsiveness. Transduced (G1TkSvNa) cells and control cells were cultured in IL-2 with (● and ▲, respectively) or without (○ and △, respectively) GCV (1 μg/mL). The percentage of remaining cells (A) and the proliferative response to IL-2 (B) were measured 7 and 14 days after initiation of the culture with or without GCV.
geneic response of transduced cells resulting in CPM counts of 1 to 10. GCV treatment specifically inhibited the alloreactivity (Fig 6A), with proliferation indexes ranging from 1 to 10. As noted previously with PHA-stimulated cells, this allogeneic response was specifically inhibited in the transduced cells by GCV treatment.

To further examine this question, we transduced a CD4+ mature T-cell lymphoma cell line (HUT-78) by coculture of the cells with the PA317/GITKSvNa packaging cell line followed by G418 selection. We then tested the effects of GCV on cell growth using various combinations (0% to 100%) of transduced and nontransduced T cells (Table 2). As with primary T cells, we found no evidence in favor of a significant bystander effect. The level of GCV-induced growth inhibition was proportional to the percentage of tk-HUT-78 cells present in the well and always less than the expected GCV-induced growth inhibition in the absence of a bystander effect.

The absence of an HS-tk-mediated bystander effect in cultured T cells and HUT-78 tumor cells strongly suggests that most if not all of the IL-2-responsive cells after 1 week of G418 selection were indeed expressing the HS-tk.

Table 2. Effect of GCV Treatment on the IL-2-Induced Proliferation of a Mixture of Transduced (50%) and Nontransduced (50%) T Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>G418 (μg/ml)</th>
<th>50% Control T Cells + 50% transduced T Cells</th>
<th>GCV</th>
<th>Observed CPM</th>
<th>Theoretical CPM if No Bystander Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>214.2 ± 18.5 (68)</td>
<td></td>
<td>178.5 ± 7.3 (17)</td>
<td>191.0 (9)</td>
</tr>
<tr>
<td>TK</td>
<td>0</td>
<td>192.1 ± 23.6 (26)</td>
<td></td>
<td>216.7 ± 17.4 (21)</td>
<td>188.7 (16)</td>
</tr>
<tr>
<td>TK</td>
<td>600</td>
<td>111.2 ± 10.8 (86)</td>
<td></td>
<td>173.9 ± 20 (28)</td>
<td>117.9 (33)</td>
</tr>
<tr>
<td>TK-LSM</td>
<td>0</td>
<td>166.5 ± 10.6 (88)</td>
<td></td>
<td>188.8 ± 18.1 (25)</td>
<td>120.3 (41)</td>
</tr>
<tr>
<td>TK-LSM</td>
<td>600</td>
<td>103.7 ± 2 (85)</td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

The theoretical GCV-induced growth inhibition in the absence of a bystander effect:

\[
\text{Observed CPM} = \frac{[50\% \text{ CPM TK Cells} + \text{GCV} + 50\% \text{ CPM Control Cells} + \text{GCV}] \times 100}{50\% \text{ CPM TK Cells} + 50\% \text{ CPM Control Cells}}
\]

Percentages are in parentheses.

Abbreviation: NA, not applicable.

bystander cell killing has been recently described in various tumor cell models. Therefore, it was possible that only a minority of the IL-2-responsive cells were expressing HS-tk and that a "bystander" effect was responsible for GCV-induced growth inhibition of nontransduced T cells. To address this possibility, we examined the effects of GCV in a mixture of transduced and nontransduced T cells (50% of each) and found no evidence for an HS-tk-mediated bystander effect in T cells (Table 2). Indeed, the level of GCV-induced growth inhibition was never greater than the growth inhibition expected with the same amount of transduced or nontransduced cells cultured separately as one would have expected if there had been a bystander effect.

To further examine this, we transduced a CD4+ mature T-cell lymphoma cell line (HUT-78) by coculture of the cells with the PA317/GITKSvNa packaging cell line followed by G418 selection. We then tested the effects of GCV on cell growth using various combinations (0% to 100%) of transduced and nontransduced HUT-78 cells (Fig 5). As with primary T cells, we found no evidence in favor of a significant bystander effect. The level of GCV-induced growth inhibition was proportional to the percentage of tk-HUT-78 cells present in the well and always less than the expected GCV-induced growth inhibition in the absence of a bystander effect.

The absence of an HS-tk-mediated bystander effect in cultured T cells and HUT-78 tumor cells strongly suggests that most if not all of the IL-2-responsive cells after 1 week of G418 selection were indeed expressing the HS-tk.

Alloreactivity of the primary T cells after gene transfer and G418 selection is inhibited by GCV treatment. In most cases, HS-tk/NeoR gene transfer and G418 selection of PHA-then IL-2-stimulated T cells resulted in cells capable of alloreactivity (Fig 6A), with proliferation indexes ranging from 1 to 10. GCV treatment specifically inhibited the allogeneic response of transduced cells resulting in CPM counts similar to the counts of transduced cells with no allogeneic stimulus. Control cells selected in G418 were unable to generate a significant alloresponse (Fig 6A).

We next examined the effects of GCV on the allogeneic response of transduced G418-selected mononuclear cells that had been initially stimulated with allogeneic cells from the same donor instead of PHA (Fig 6B). In the present case, the allogeneic response of the transduced cells was strong. As noted previously with PHA-stimulated cells, this allogeneic response was specifically inhibited in the transduced cells by GCV treatment (84% growth inhibition). In contrast, the control nontransduced cells exhibited higher preallostim-
Fig 6. GCV-induced growth inhibition of alloreactive HS-tk-transduced (GlTkSvNa) T cells. Peripheral mononuclear cells were stimulated with PHA (A) or with irradiated allogeneic cells + IL-2 (500 U/mL) at day 2 (B) before being transduced and selected in G418 (500 μg/mL) and IL-2 (500 U/mL) for 6 days. The surviving cells were then washed, adjusted, and plated with allogeneic target cells and low-dose IL-2 (50 U/mL), with or without GCV (1 μg/mL), for 5 days. In the case of initial allostimulation before transduction (Fig 4B), the same allogeneic target cells were used (transduced and G418-selected cells, ○; nontransduced and G418-selected cells, △; nontransduced and non-G418-selected cells, ◦).

**DISCUSSION**

Acute and chronic GVHD remain a significant source of mortality and morbidity after allogeneic BMT. The only efficient way of reproducibly preventing GVHD, ie, ex vivo marrow graft TCD, is associated with enhanced graft rejection and the loss of the GVL effect. Furthermore, non-TCD BMT recipients will benefit from a GVL effect even in the absence of GVHD. Unfortunately, there are no reliable ways of predicting the risk of subsequent GVHD occurrence and severity. Because of similar results in terms of survival, both non-TCD BMT as well as TCD-BMT are presently used for the treatment of a variety of diseases. However, innovative therapeutic strategies for the control of post-BMT alloreactivity are urgently needed especially at a time when, in an effort to increase donor availability, BMT from partially matched related or matched unrelated donors are being evaluated.

The approach we have developed and report here has the potential of overcoming some of the difficulties in attempting to square the circle of post-BMT alloreactivity, ie, (1) preservation of the GVL and graft-enhancing effects of donor T cells early after BMT and throughout the postransplantation period for patients not experiencing severe GVH and (2) increasing the specificity of anti-GVHD treatment with therapy-induced immunosuppression restricted to the mature donor T-cell transferred with the allogeneic hematopoietic stem cells.

In the present studies, we have established that retroviral-mediated transfer of HS-tk and NeoR into primary human T lymphocytes is possible and that G418 selection does indeed result in T cells specifically inhibited by GCV at concentrations achievable in vivo. Our data also indicate that, compared with control cells, target cells exposed to gene transfer and subsequent G418 selection have a lower proliferative response to IL-2. Because the different cell populations in our experiments were adjusted for “live” (trypan blue exclusion) cells before being plated with IL-2, this finding suggests that, among the cells surviving 7 days of culture in G418, only a fraction of the “live” cells are capable of a proliferative response and that a substantial proportion of the cells (although still alive) are severely damaged by G418. This hypothesis is further substantiated by the following observations: (1) a fraction of the cells not exposed to HS-tk and NeoR gene transfer are still alive after growing in G418 for 1 week but these cells were incapable of responding.
HS-tk EXPRESSING PRIMARY T LYMPHOCYTES

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FUNCTION BUT ALSO ALLOW THE DEVELOPMENT IN VIVO OF A FUNCTIONAL IMMUNE REPertoire WITH THE RECONSTITUTION OF SPECIFIC IMMUNE FUNCTIONS (SUCH AS A PROLIFERATIVE RESPONSE TO ALLOANTIGENS, TETANUS TOXOID, AND VIRA VIRAL ANTIGENS).31 OTHER STUDIES WITH NEO-tk-TRANSduced TUMOR-INFILTRATING LYMPHOCYTES (TILs) HAVE SUGGESTED THAT THE TRANSDED POPULATIONS WERE REPRESENTATIVE OF THE TOTAL TIL POPULATION IN TERMS OF THE T-CELL RECEPTOR REPERTOIRE AS WELL AS mRNA CYTOKINE PATTERN24 AND THAT THE PHENOTYPE AND CYTOTOXICITY PROFILE OF THE GENE-TRANSduced TILs WAS SIMILAR TO THOSE OF NAIVE TILs.33 THESE VARIOUS STUDIES THEREFORE SUGGEST THAT, AFTER EX VIVO GENE TRANSFER AND CULTURE IN G418 AND IL-2, TRANSduced HUMAN T LYMPHOCYTES WILL MAINTAIN IN VIVO SPECIFIC IMMUNE FUNCTIONS SUCH AS ALLOREACTIVITY AND GVL EFFECTS.

BECAUSE CHRONIC GVHD CAN OCCUR LATE AFTER BMT, IN VIVO DEPLETION OF THE HS-tk-TRANSduced T CELLS HAS TO BE POSSIBLE FOR SEVERAL MONTHS AFTER BMT. STABLE HS-tk EXPRESSION IS THEREFORE OF CRUCIAL IMPORTANCE. IN THE PRESENT EXPERIMENTS, GCV-INDUCED GROWTH INHIBITION OF HS-tk-TRANSduced HUT-78 CELLS WAS UNALTERED OVER AN 80-DAY PERIOD OF GROWTH IN THE ABSENCE OF G418, AND IN FACT, WAS NOT DIFFERENT THAN THE GCV-INDUCED GROWTH INHIBITION OF TRANSduced CELLS MAINTAINED IN G418 UP TO THE END OF THE EXPERIMENT. A SIMILAR EXPERIMENT WITH NON-AG-SPECIFIC PRIMARY T LYMPHOCYTES IS NOT EASILY PERFORMED. HOWEVER, WE HAVE EXAMINED Tk EXPRESSION IN PRIMARY T CELLS UP TO 15 DAYS AFTER THE END OF G418 SELECTION WITHOUT EVIDENCE FOR ANY LOSS OF GCV SENSITIVITY (DATA NOT SHOWN). FURTHERMORE, IN VIVO DATA ARE NOW AVAILABLE IN HUMANS SUGGESTING THAT EXPRESSION IN LYMPHOCYTES OF THE ADA GENE UNDER THE Control OF THE SAME LTR SEQUENCES IS DURABLE (>6 MONTHS).34 IN VIVO EXPERIMENTS OF HUMAN T CELLS GROWN IN SCID MICE ARE ALSO PRESENTLY UNDERWAY TO FURTHER ADDRESS THIS ISSUE.

OTHER THAN IN ALLOGENIC BMT, THE USE OF ALLOGENIC HS-tk-TRANSduced PRIMARY T LYMPHOCYTES COULD BE OF BENEFIT IN SEVERAL OTHER CLINICAL SETTINGS. THE TRANSFUSION OF DONOR T CELLS IN PATIENTS WITH RELAPSED CHRONIC MYELOGENOUS LEUKEMIA AFTER ALLOGENIC BMT MEDIATES A POTENT ANTILEUKEMIC EFFECT WITH, UNFORTUNATELY, AN EXPECTED SIDE EFFECT—MODERATE TO SEVERE GVHD.35,36 GCV-MEDIATED IN VIVO MODULATION OF HS-tk-TRANSduced DONOR-DERIVED T CELLS IN THIS SETTING COULD BE OF INTEREST. ALSO, THE POTENTIAL OF INDUCING A GVL EFFECT AFTER AUTOLOGOUS BMT BY ADMINISTRATING ALLOGENIC T CELLS WITH OR WITHOUT IL-2 IS BEING EVALUATED27,38 AND HERE, TOO, SPECIFIC IN VIVO CONTROL OF THE T CELLS WOULD BE BENEFICIAL.

IN SUMMARY, WE HAVE DEMONSTRATED THAT HS-tk—EXPRESSING PRIMARY T LYMPHOCYTES CAN BE GENERATED AND THAT GCV CAN SPECIFICALLY INHIBIT THEIR ACTIVITY, THEREFORE ESTABLISHING THE RATIONALE AND FEASIBILITY OF SUCH AN APPROACH FOR THE IN VIVO CONTROL OF DONOR T CELLS TRANSFERRED WITH AN ALLOGENIC GRAFT. DRAWING ON THE INFORMATION GAINED IN THE PRESENT STUDY, WE WILL SOON INITIATING A PHASE I CLINICAL TRIAL IN ALLOGENIC BMT.

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