Suicide Gene Therapy for Plasma Cell Tumors

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Suicide gene therapy for plasma cell tumors was attempted in severe combined immunodeficient (SCID) mice injected with human myeloma cell lines. Initially, a ganciclovir-induced bystander effect was observed in vitro using myeloma cells transduced with a herpes simplex thymidine kinase (HSVtk) gene. Transduced cells injected subcutaneously (SC) into SCID mice could be eradicated by the administration of ganciclovir (GCV). Furthermore, an in vivo bystander effect was noticed when mice received mixtures of HSVtk-positive and nontransduced cells. Unexpectedly, a “distant bystander” effect was observed as tumors in regions inoculated with only nontransduced cells were significantly smaller and had increased frequency of apoptotic figures and decreased mitotic frequency in GCV-treated mice transplanted with HSVtk-positive cells at a different region compared with control mice.

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MULTIPLE MYELOMA is a hematologic malignancy resulting from the uncontrolled proliferation of a single clone of plasma cells in the bone marrow. Chemotherapy for multiple myeloma has been used for almost three decades, but the disease is still considered as essentially incurable despite recent progress with high-dose therapy and bone marrow transplantation.1,2

Since the initial description of the severe combined immunodeficient (SCID) mice3 and the original studies related to the engraftment of human lymphoid cells in these mice,4 several investigators have reported SCID mouse models of human hematologic malignancies including lymphomas and leukemias.5–12 Recently, in vivo models for human multiple myeloma were established in SCID mice by injecting myeloma cell lines5,13 or freshly obtained bone marrow cells from multiple myeloma patients.14,15 In particular, the engraftment of the ARH-77 myeloma cell line in SCID mice resembled the growth of multiple myeloma in humans,15 which gives the opportunity of studying novel therapeutic approaches for human multiple myeloma.

Several proposals for the gene therapy of cancer have been made that involve transfer of the therapeutic gene either into the tumor cells directly or into the immune cells to enhance their presumed tumoricidal properties.16–21 Transfer of the herpes simplex thymidine kinase gene (HSVtk) into the tumor cells using retroviral or adenovirus vectors followed by the administration of ganciclovir (GCV) provides a potential strategy for the treatment of some malignancies.22–27 GCV is metabolized by the viral thymidine kinase into a monophosphate form and subsequently to GCV-triphosphate by endogenous mammalian kinases. GCV-triphosphate is a potent inhibitor of viral DNA-polymerase and a purine analogue competing with normal nucleotides for viral DNA replication.28 In addition, GCV-triphosphate competes with normal nucleotides for DNA replication in mammalian cells and can cause cell growth inhibition and cell death.29 Therefore, cells that express HSVtk become sensitive to the toxic effect of GCV and can be eradicated in vivo by the administration of GCV. However, during GCV treatment, not only the cells that express HSVtk gene are killed, but also frequently neighboring tumor cells that are not genetically altered.30 This phenomenon is called “bystander effect”. Although tumor killing by the bystander effect has been obtained in different malignant tumors, it has not been reported in hematologic malignancies. In this study, we demonstrate that a bystander effect can be obtained both in vitro and in vivo in myeloma cells indicating the potential application of virus-directed enzyme/prodrug therapy in multiple myeloma or plasmacytoma.

MATERIALS AND METHODS

Cell lines. The human myeloma cell lines ARH-77 (IgG1 with kappa light chain) and RPMI 8226 (lambda light chain) were obtained from the American Type Culture Collection (ATCC CRL 1621 and ATCC CCL 155, Rockville, MD). Cells were maintained in RPMI-1640 ( Gibco, Paisley, UK) supplemented with 100 μmol/L L-glutamine (GIBCO), 10% fetal calf serum (FCS; Gibco), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Gibco).

Retroviral vector. PLTN 3.3, a replication deficient retroviral vector, containing the bacterial neomycin resistance (NeoR) and HSVtk fusion gene, was kindly provided as a high titer PA317 amphotropic vector-producing fibroblast (VFP) clone by Dr Michael Blase (National Institutes of Health [NIH], Bethesda, MD). To produce virus-containing supernatant, the PA317 VFP were plated in 175 cm² flasks in Dulbecco’s modified Eagle’s medium with high glucose (DMEM; Gibco) and 10% heat-inactivated FCS. The medium was changed when cells got subconfluent, then collected after 24 hours, filtered (0.45 μm) and frozen at –70°C. The titer of supernatant was 10⁷/mL as measured on 3T3 cells. The supernatant containing the viral particles was negative for helper virus when target 3T3 cells were tested by polymerase chain reaction (PCR) for amphotropic env sequences as previously described.29

Transduction of myeloma cell lines. One million ARH-77 or RPMI 8226 cells were incubated in supernatant containing the viral particles (5 virus particles per cell) with 8 μg/mL polybrene (Sigma, St Louis, MO) for 3 hours. Thereafter, the supernatant was replaced with the basal medium consisting of RPMI-1640 and 10% FCS. The same transduction procedure was repeated daily for 2 more consecutive days. The cells were then cultured for 24 hours in me-
dium and exposed to 1 mg/mL G418 (Gibco). Resistant cells were selected after 2 weeks of exposure to G418.

In vitro GCV sensitivity assay. To determine the optimal cytotoxic effect of GCV, transduced and nontransduced cells were incubated at a concentration of 3 × 10^5 cells in 1 mL of the basal medium in polystyrene round-bottom tubes (Falcon, Plymouth, UK). After 24 hours incubation, GCV (Syntex, Palo Alto, CA) was added to a final concentration of 1 to 50 μg/mL. The GCV containing medium was changed daily for 2 weeks, after which living cells were counted using the trypan blue dye exclusion test.

In vitro analysis of the bystander effect. To determine the effect of HSVtk-positive cells on HSVtk-negative cells in vitro, experiments were performed in which HSVtk-positive and -negative cells were mixed at different ratios. Mixtures of cells were incubated at a concentration of 3 × 10^5 cells in polystyrene round-bottom tubes (Falcon) in basal medium and 10 μg/mL GCV. Medium was changed daily for 2 weeks and thereafter viable cells were counted using the trypan blue dye exclusion test.

Mice. The C.B-17 scid/scid mutant (SCID) mice used in these experiments were bred and maintained under specific-pathogen-free conditions in the facilties of Huddinge Hospital. Cages, water bottles, and bedding were autoclaved and changed twice a week. All procedures involving animal use were approved by the local ethical committee.

Establishment of tumors in SCID mice. Before inoculation into mice, ARH-77 or RPMI 8226 cells were washed twice with phosphate-buffered saline (PBS). To establish tumors, 10^7 cells were injected subcutaneously (SC) on the back of SCID mice. Animals were examined daily until tumors became palpable, thereafter the diameter, in two dimensions, was measured twice a week using callipers. Animals were killed and dissected 6 weeks after transfer of cells.

The percentage of HSVtk-positive cells required for tumor regression was determined by SC injection of a mixture of HSVtk-positive and -negative ARH-77 cells in different proportions: (1) 100% HSVtk-negative cells; (2) 100% HSVtk-positive cells; (3) 50% each of HSVtk-negative and -positive cells; and (4) 75% HSVtk-negative and 25% HSVtk-positive cells. The cells were injected SC into four separate locations on the back of each mouse.

Kinetic assays for growth of ARH-77 cells. In the absence of GCV, 3 × 10^5 transduced or nontransduced cells were separately cultured in basal medium in six-well dishes for 2 weeks. Cells were counted using the trypan blue dye exclusion test after 2 weeks of incubation. For in vivo growth kinetics, 10^7 transduced or nontransduced cells were injected SC into four separate locations on the back of each mouse. Tumor size was measured using callipers 6 weeks after transfer of cells.

After lethal irradiation of nontransduced ARH-77 cells with 100 Gy, 3 × 10^5 irradiated cells and 3 × 10^5 nonirradiated cells were cultured either separately or as a mixture for 2 weeks in basal medium. After 2 weeks of incubation, cells were counted using the trypan blue dye exclusion test. In in vivo experiments, 10^5 irradiated cells and 10^7 nonirradiated cells were injected SC either separately or as a mixture into four separate locations on the back of each mouse. Tumor size was measured using callipers 6 weeks after transfer of cells.

GCV treatment. One week after SC injection of cells, the mice were given GCV twice a day by intraperitoneal (IP) injections of: (1) 15 mg/kg for 2 weeks; (2) 15 mg/kg for 3 weeks; (3) 150 mg/kg for 5 days; or (4) 150 mg/kg for 2 weeks. Mice in the control group were injected IP with a similar volume of saline. To evaluate the direct effect of GCV on nontransduced cells, 10^7 nontransduced ARH-77 cells per site were injected SC into four different locations on the back of each mouse. One week later, mice were given twice a day IP injections of either saline or 15 mg/kg GCV for 3 weeks.

To see whether nontransduced cells in the mixture of transduced and nontransduced populations could create tumor masses in the absence of a bystander effect, only 5 × 10^9 parental ARH-77 cells were injected SC into four separate locations on the back of each mouse. The tumor size 6 weeks after cell transfer were compared in control and GCV-treated groups.

DNA isolation and PCR. Isolation of DNA was performed using sodium dodecyl sulfate (SDS) proteinase-K lysis of cells followed by phenol/chloroform extraction. PCR reactions were performed using 100 ng of each primer and 0.2 U of Taq polymerase (BRL, Life Technologies, Paisley, UK) in a total volume of 50 μL. Samples were incubated initially for 5 minutes at 95°C followed by 30 cycles using primers neo1 or neo5 and primers Ava I or Ava II. The presence of neoR gene sequence in the DNA samples resulted in a 790-bp fragment when a combination of neo1-neo5 primers were used.

Histological analysis. At necropsy, subcutaneous tumors were excised from GCV-treated as well as control animals. Tumors were stained with hematoxylin and eosin for morphological analysis. Using the NIH image program, the total tumor area of representative sections, as well as the area of necrosis within the tumors, was analyzed and expressed in arbitrary units. Within nonnecrotic homogeneous tumor areas, the rim of the tumors, the number of mitotic figures and apoptotic cells was assessed in a minimum of 15 randomly chosen areas.

Enzyme-linked immunosorbent assay (ELISA) and serum electrophoresis. Human IgG was detected by serum electrophoresis of the samples obtained from mice injected with ARH-77 cells. Using conventional methods, 4 μL of undiluted serum samples from repopulated SCID mice was used for electrophoresis. Rabbit Ig to human IgG (Dako) was used for immunofixation. Human IgG in the same samples was also quantified by sandwich ELISA using rabbit anti-human IgG (Dakopatts) as the capture agent and alkaline phosphatase-conjugated affinity-purified rabbit anti-human IgG (Dakopatts) for detection.

Statistical analysis. The Chi square test was used to compare the tumor size as well as the presence of macroscopic tumor necrosis between the control and GCV-treated mice. The Wilcoxon-Mann-Whitney test was used to compare the effect of GCV and saline on tumors derived from nontransduced cells. The t-test was used to compare the growth kinetics of transduced and nontransduced cells in vitro and in vivo, as well as to compare the in vitro and in vivo effects of lethally irradiated ARH-77 cells on parental nonirradiated cells. To compare histological differences in tumors developed in various groups, statistical analysis was performed using the JMP program (SAS Institute Inc, Cary, NC) applying the Dunnett's method. The levels are expressed as mean ± standard deviation (SD). Differences were considered significant when probability values were < 0.05.

RESULTS

HSVtk transduction of the myeloma cell lines. The transduction rate after 2 weeks of G418 selection was 13% and 9% for ARH-77 and RPMI 8226, respectively. Genomic analysis of transduced cells by PCR confirmed the presence of the transgene. NeoR resistant cells positive for HSVtk were selected by G418. To optimize the concentration of GCV, transduced and nontransduced ARH-77 cells were grown in medium containing GCV at various concentrations ranging from 1 to 50 μg/mL and cell viability was assayed.
In vitro effect of ganciclovir treatment on ARH-77 HSVtk-negative (■) and HSVtk-positive (□) cells. A total of 3 \times 10^6 cells were incubated for 2 weeks with various concentrations of GCV. After 14 days, as shown in Fig 1, tumor cells that contained the HSVtk gene were sensitive to the GCV treatment at concentrations down to 5 \mu g/mL, whereas optimal selection was obtained at a concentration of 10 to 20 \mu g/mL. The optimal dose of GCV to kill transduced RPMI 8226 was similar to that found for ARH-77 cells. A toxic effect of GCV on nontransduced cells was observed at a concentration of 50 \mu g/mL.

In vitro and in vivo growth kinetics of ARH-77 cells. Experiments were performed to evaluate growth kinetics of transduced and nontransduced ARH-77 cells. There was no statistically significant difference in the number of cells after 2 weeks of incubation in basal medium between transduced versus nontransduced cells (3.06 \times 10^6 \pm 0.09 \times 10^6, 3.01 \times 10^6 \pm 0.12 \times 10^6, respectively). In addition, no significant difference was found in the size of SC tumors when the same number of either transduced or nontransduced cells were injected into SCID mice (data not shown).

In vitro bystander effect. To investigate putative in vitro bystander effects, experiments were performed in which ARH-77 HSVtk-positive and -negative cells were mixed in different proportions. When 50\% of the mixture consisted of HSVtk-positive cells, the majority of cells were killed within 2 weeks of exposure to GCV (Fig 2), indicating that HSVtk-positive myeloma cells could exert an in vitro bystander effect. Transduced RPMI 8226 cells were killed after GCV exposure but there were no indications of an in vitro bystander effect (data not shown).

Furthermore, experiments were performed to see if the presence of dead cells in the nontransduced cell mixture per se could exert any in vitro bystander effect. After testing with various doses of irradiation, 100 Gy was found to be a lethal dose for ARH-77 cells. There was no effect of dead cells on the number of neighboring living cells after 2 weeks of incubation in vitro (3.00 \times 10^6 \pm 0.12 \times 10^6 and 3.02 \times 10^6 \pm 0.13 \times 10^6, respectively).

In vivo bystander effect. SC tumors could be generated from both ARH-77 and RPMI 8226 human myeloma cell lines in SCID mice. Tumors developed in all animals (n = 5) injected with 10^7 RFMI 8226 cells and in all animals (n = 5) injected with 10^7 ARH-77 cells. In each group, tumors became palpable 19 to 33 and 7 to 12 days after injection of cells, respectively. Tumors generated from RPMI 8226 cells grew slowly and varied more in size in comparison to the ARH-77 cells; tumor diameter 6 weeks after injection of cells were 21 \pm 9.6 and 10.2 \pm 7 mm (mean \pm SD), respectively. Therefore, the ARH-77 cell line was chosen for the subsequent experiments.

To determine whether the in vitro bystander effect could also be obtained in an in vivo situation, transduced and nontransduced ARH-77 cells were injected into the SCID mice in various proportions followed by the administration of GCV. Four of 17 mice in the GCV group had small palpable tumors even before initiation of the GCV treatment 1 week after injection of the cells. The size of the tumor masses at the end of the experiment, 6 weeks after cell injection, are summarized in Table 1. All animals in the control group (n = 8) developed large tumors at all injection sites in each mouse regardless of the proportion of HSVtk-negative or positive cells in the injected mixture. Furthermore, all GCV-treated mice (n = 17) developed tumors at locations inoculated with HSVtk-negative cells only. However, 14 of 17 (82\%) mice receiving GCV treatment had no tumors at the...
inoculation site when only HSVtk-positive cells were used. In 12 of 17 (70%) animals, no tumors were seen at the inoculation site when a mixture of 50% HSVtk-negative and HSVtk-positive cells was used. Three of 17 (17%) animals had no tumors at the inoculation site when a mixture of 75% HSVtk-negative and 25% HSVtk-positive cells was used. Necrotic skin ulcerations were seen in the tumors consisting of a 75% HSVtk-negative and 25% HSVtk-positive cell mixture, although the size of these tumors was smaller than the tumors derived from nontransduced cells only. Small tumor masses appeared in all animals (n = 3) that received only 5 days of high-dose GCV treatment. Some representative mice from this experiment are shown in Fig 3.

It could be argued that the residual 5 × 10^6 nontransduced cells in the mixture would be unable to form a tumor mass. However, tumor masses developed in all mice injected with only 5 × 10^6 parental ARH-77 cells with (n = 6) or without (n = 3) GCV treatment; tumor diameters 6 weeks after injection of cells were 12.8 ± 1.6 mm (mean ± SD). No statistically significant difference was found in tumor size between GCV-treated and control mice.

Experiments were also performed to see if the presence of dead cells in the nontransduced cells mixture per se could exert any in vivo bystander effect. Cells were irradiated with 100 Gy. No statistically significant difference was found in tumor size between animals receiving only living cells or those injected with a mixture of living and irradiated cells.

"Distant bystander" effect. Ten of 12 (83%) tumors derived from nontransduced cells in mice also injected with HSVtk-positive cells, but in a different region, developed macroscopically detectable necrotic lesions after a 2-week high or 3-week low dose of GCV. In the control groups treated with saline, only five of 20 (25%) tumors displayed necrotic lesions (P < .01). Furthermore, as shown in Table 1, the size of the tumors derived from nontransduced cells in mice treated with a low-dose of GCV for 3 weeks or with a high-dose for 2 weeks was significantly smaller than those in control mice (P < .01). The mechanism underlying this phenomenon is not known, but we have tentatively called it a "distant bystander" effect.

Effect of GCV on nontransduced cells. To rule out the effect of GCV treatment per se on tumor formation, eight mice were injected SC with 10^7 nontransduced cells into four separate locations on the back and were given either saline or low-dose GCV (15 mg/kg twice a day) for 3 weeks. Six weeks after cell injection, no significant difference was found between the size of tumors appearing in the control and GCV-treated mice (Fig 4). Furthermore, no difference was found comparing the size of tumors in different injection sites in each animal. A total of three of 32 (9%) tumor masses in this experiment, two in the control group and one in the treatment group, were associated with skin ulcers similar to those found in the GCV-treated mice transplanted with HSVtk-positive cells. No side effects of GCV were noticed in mice treated with the low-dose for 2 weeks or with the high-dose for 5 days. However, mice treated with high-dose of GCV for 2 weeks showed weight loss and weakness.

Human IgG production in SCID mice. In seven mice injected with 10^7 ARH-77 cells, the presence of human IgG in the serum 6 weeks after cell injection was demonstrated by a distinct band on serum electrophoresis. All the mice examined had visible tumors at the time of investigation. Quantification by ELISA showed serum human IgG with a mean of 1.6 mg/mL (range, 0.6 to 2.9 mg/mL).

Histological comparisons of tumors derived from nontransduced cells. At autopsy 6 weeks after cell injection, no metastases were seen in any organs, but local tumor infiltration into adjacent muscular tissues was evident in most of the animals. Mitotic cell frequencies were investigated in the tumors derived from nontransduced cells to further study the distant bystander effect. Analysis of mitotic cell frequency within nonnecrotic homogeneous tumor areas from the different treatment groups showed that the number of mitotic cells per unit area was significantly higher in the untreated control mice (6.42 ± 2.97) than in both the high- (3.58 ± 1.73) and low- (4.01 ± 2.15) dose GCV-treated groups (Fig 5). When comparing apoptotic figures per unit area, the frequency seen in the tumors from untreated control mice was significantly lower (3.75 ± 2.09) than that in the high- (13.08 ± 11.12) and low- (9.09 ± 3.67) dose GCV-treated groups (Fig 5). There was no significant difference
in either mitotic or apoptotic cell frequency in tumors from the high- as compared with the low-dose GCV-treated group.

Two types of necrotic lesions were present: (1) well demarcated geographic areas of ischemic necrosis containing ghosts of tumor cells and surrounded by rims of necrobiosis and rich in apoptotic cell nuclei; (2) areas of eosinophilic fibrin-like necroses with rims of neutrophils. The former lesions were predominantly located in the center of the tumors, whereas the latter were associated with skin ulcers in superficial parts of the tumors. The area of tumor necrosis seen in histological sections was not significantly changed by GCV treatment. The morphology of the nucleus and cytoplasm of well preserved tumor cells was indistinguishable in tumors of untreated, low-dose, and high-dose GCV-treated mice.

DISCUSSION

Gene therapy for cancer comprises several different approaches, such as potentiating immunity of tumor cells, in
Introducing tumor suppressor genes into malignant cells with mutations in these genes and transfer of suicide genes. In this study we have attempted to use HSVtk to eradicate human myeloma tumors generated in SCID mice.

Engraftment of myeloma cell lines, in particular, ARH-77 cells, was easily obtained in SCID mice following SC transfer of cells. Apart from tumor growth at the site of injection, serum human IgG was detected in transplanted animals at levels similar to those found after IP reconstitution of SCID mice with human peripheral blood cells.

An in vivo effect of GCV on HSVtk transduced fibroblast lines has previously been reported. In mice transgenic for HSVtk, lymphoid tissues expressing HSVtk from an immunoglobulin promoter, as well as virally-induced lymphomas, were sensitive to GCV treatment. Also HSVtk-containing melanoma and gastric cell lines were sensitive to GCV in vivo.

Bystander effects have previously been reported in vivo using the transfer of retroviral vector producing cell lines transducing glioma cells in the brain as well as in colon carcinoma cells transduced with HSVtk before inoculation. Use of recombinant adenovirus for transfer of this gene to mesotheliomas also demonstrated a bystander effect in vitro at a ratio of 1:10 transduced versus nontransduced cells. In a murine sarcoma cell model using retroviral delivery, a pronounced in vivo bystander effect was only observed using 50% transduced cells, whereas in vitro, 10% transduced cells were sufficient. In other experiments, in vivo administration of retroviral vectors expressing tissue-specific HSVtk demonstrated an effect on inoculated melanoma cells. The results of the present study provide evidence for an in vivo bystander effect when HSVtk-positive cells constitute 25% of the injected myeloma cells generating subcutaneous tumors in the SCID mice.

Apart from the transgenic mice where an effect of HSVtk on lymphoid cells was seen, a suicide function of this gene in hematopoietic cells has only been investigated in an attempt to eradicate T cells exerting a graft-versus-host effect. However, in the first instance, when analyzing lymphoma cells, all cells carried the transgene and in the second, a bystander effect was not seen. Thus, to our knowledge our findings using a plasma cell tumor model constitutes the first example of a suicide gene bystander effect in hematopoietic cells.

It has previously been suggested that gap junction formation is important for a bystander effect, but endocytosis of toxic remnants from cells carrying an HSVtk gene is an alternative explanation. The level of one important protein involved in gap junction formation, connexin-43, was analyzed by Western blotting in ARH-77 and RPMI 8226 myeloma cell lines, but connexin-43 was undetectable in both transduced and nontransduced cells (data not shown). Presently, it is not clear whether other connexins are involved in gap junction formation in the ARH-77 cell line or if this represents a non gap junction-mediated bystander effect. We also do not know whether RPMI 8226, which did not exert any bystander effect in vitro, forms any gap junction. This is presently being investigated, but as there are a large number of connexins forming functionally different junctions, careful investigations are needed to resolve this issue.

The importance of an immune response has been observed in some tumor models with suicide genes. Thus, it is possible that an immune response could also be effective for plasma cell tumors, but because such a response would not appear in SCID mice, we have not, as yet, addressed this issue. SCID mice exhibit a severe immunodeficiency involving both T and B cells. It is known, however, that spleens of SCID mice contain mature natural killer (NK) cells that are phenotypically and functionally indistinguishable from NK cells found in normal mice. Therefore, these mice may still be able to initiate an immune response against transferred tumor cells.

Another factor that might contribute is endothelial cell damage resulting in destruction of local vasculature and reducing the blood supply. However, as ischemic necrosis in the central parts of the tumors were not significantly increased in GCV-treated as compared with untreated control animals, it seems unlikely that the tumor inhibiting effect of GCV would be exerted through the action of cytotoxic metabolites on endothelial cells leading to thrombosis. Rather the central necrosis, often seen in experimental tumor system, could result from insufficient vascularization in the rapidly growing tumor.

The results from the morphological analysis suggest that a soluble factor might contribute to the difference in size of tumors derived from nontransduced cells in the GCV-treated group as compared with the saline-treated animals. This could be caused by interference of treatment with the turnover of tumor cells indicating a "distant bystander" effect or lower growth/survival promoting effect. However, we were unable to observe any direct effect of GCV treatment on growth of tumors derived from nontransduced ARH-77 cells.
Sera from GCV-treated and control animals, as well as nonreconstituted SCID mice, were added to in vitro cultures of ARH-77 cells. Addition of 10% plasma from all groups reduced cell growth as compared with growth in medium containing only FCS. However, there was no statistically significant difference in this inhibitory effect between plasma obtained from GCV-treated, control, or nonreconstituted SCID mice (data not shown).

To see whether any general inflammatory reaction was involved in the "distant bystander", mouse and human tumor necrosis factor-α (TNF-α) levels were measured in mouse sera obtained at autopsy. There was no difference in the TNF-α levels irrespective of the presence of tumor or treatment given as compared with untreated control SCID mice (data not shown). However, we cannot exclude that increased levels were present at other time points. Programmed cell death involving the cell surface antigen Fas constitutes yet another possibility, as ARH-77 cells have recently been found to both express Fas antigen and go through apoptosis following its cross-linking.43

Fig 5. Distant bystander effect in animals receiving non-transduced and transduced ARH-77 cells in different locations. Tumors were recovered from locations injected with only non-transduced cells. (A) Photomicrograph (x 280) of a subcutaneous tumor of ARH-77 cells in a SCID mouse without ganciclovir treatment. Tumor shows frequent mitotic figures (open arrows) and occasional apoptotic cells (filled arrow). (B) Photomicrograph (x 280) of a subcutaneous tumor of ARH-77 cells from a SCID mouse treated with ganciclovir. The tumor shows a few mitotic cells (open arrows) and frequent apoptotic figures (filled arrows).

It is difficult to foresee whether the bystander effect can be used clinically in the treatment of plasmacytoma or multiple myeloma. It may be feasible to innoculate autologus in vitro-transduced myeloma cells44 that might home to adjacent in vivo-persistent myeloma cells resistant to previous cytotoxic drug treatment. It may seem less likely to use established...
allogeneic cell lines such as ARH-77. However, in certain patients with resistant myeloma, such an approach could perhaps be feasible considering the high efficiency of transduction.

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