Enhancing the Efficacy of a Weak Allogeneic Melanoma Vaccine by Viral Fusogenic Membrane Glycoprotein-mediated Tumor Cell-Tumor Cell Fusion

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ABSTRACT

We have investigated how to make K1735 cells, a poor allogeneic melanoma vaccine, more effective for protection against B16 in vivo. To promote antigen release in an immunologically effective manner, tumor cells were transfected with a viral fusogenic membrane glycoprotein (vesicular stomatitis virus G glycoprotein), which kills cells through the formation, and degeneration, of large multinucleated syncyta. Vaccines consisting of a 1:1 mix of fusing allogeneic and autologous cells led to dramatic increases in survival of mice in both prophylactic and therapy models, dependent upon T cells, the mechanism of tumor-tumor cell fusion, and the nature of the fusion partner. Syncytia activate macrophages and fusogenic membrane glycoprotein-mediated cell killing very efficiently promotes cross-priming of immature dendritic cells with a model tumor antigen. Our data suggest that the unique manner in which syncytia develop and die provide a highly effective pathway for tumor antigen release and presentation to the immune system and offers a novel mechanism by which cancer cell vaccines may be prepared for clinical use.

INTRODUCTION

The identification of individual tumor-associated antigens has generated hopes that peptide vaccines may eventually be capable of immunizing patients against disease, although clinical success using these antigens as vaccines, many of which are also normal tissue-associated molecules in the case of melanoma, has yet to be shown. Therefore, vaccine candidates derived from whole cells retain considerable appeal attributable to the ability, at least in theory, to cover the entire repertoire of tumor antigens in a single vaccine preparation.

Production of patient-specific cancer vaccines is currently being addressed in clinical trials. However, for general clinical utility, it would be attractive to use stable, easily stored, and extensively characterized cell lines that are known to express at least a proportion of immunologically relevant tumor antigens. For these reasons, allogeneic tumor cell vaccines have been developed. Allogeneic cells also provide powerful adjuvant effects based on the phenomenon of alloagression in which T cells react very potently against the MHC antigens of allogeneic cells, generating a cytokine-rich environment to recruit dendritic cells, although there is some debate about the efficacy of this pathway. Allogeneic vaccines consisting of multiple cell lines with different spectra of antigen expression have been constructed and used with adenovirus (26) or genetic expression of cytokines such as GM-CSF (6). We have used the K1735 melanoma cell line, derived from C3H mice, as the platform for the allogeneic vaccine in a model of protection against the development of B16 melanoma, which is syngeneic to C57BL mice. K1735 cells, by themselves, only very poor immunogens against challenge with B16 cells in C57BL mice, a situation that is likely to be the case in clinical settings. However, under appropriate immunological conditions, they can be effective vaccines.

Previously, we have shown that expression of genes encoding viral FMGs in tumor cells leads to very potent local tumor cell killing through fusion of tumor cells to each other. The fusion event leads to the formation of large multinucleated syncyta that subsequently die through nonapoptotic, autophagic-like mechanisms that are immune-activating through induction of stress-related proteins and expression of the viral immunogens that act as potent adjuvants. Therefore, we reasoned that fusing tumor cells to each other through these immune-stimulatory mechanisms of cell killing may provide an effective method to liberate relevant tumor antigens from vaccine cells along with several additional immune-potentiating benefits. Here we demonstrate that FMG-mediated fusion of a mixture of allogeneic and autologous tumor cells was able both to activate prophylactically against challenge with parental B16 cells and to cure established tumors. Syncytial formation activated macrophages and facilitated cross-priming of immature DCs with a model ova antigen from both autologous and allogeneic cells. We propose that FMG-mediated syncytial formation mimics a pathological-type infection to which tumor cells react with activation of stress-related programs that alert the immune system to the cell death in a potient, immunostimulatory fashion.

MATERIALS AND METHODS

Cell Lines. The murine melanoma B16.F1 (4) and K1735 (45) and murine colorectal CMT93 (35) cell lines used in this study have been described previously. B16ova or NIH3T3 ova cells were derived from the parental cell lines by transduction with a cDNA encoding the ovalbumin gene. K1735 cell lines expressing cytokines or hsp70 were generated by transfection or retroviral infection with constructs as previously described expressing murine GM-CSF (46), IL-12 (47), IFN-γ, hsp70 (35), or pBabePuro (48).

Detection of Cytokine Production from Cell Lines. Cell line supernatants were assayed for cytokines by ELISA using antibody pairs obtained from PharMingen (Cambridge BioScience, Cambridge, United Kingdom). hsp70 expression was confirmed by Western Blot analysis for murine hsp70 as described previously (49). Clones selected for study produced cytokines as follows: K1735-IL-12, 8.3 ng/ml/4 × 10^5 cells/h; K1735-GM-CSF, 1 ng/ml/4 × 10^5 cells/h; and K1735-IFN-γ, 3.7 ng/ml/4 × 10^5 cells/h.

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3 The abbreviations used are: GM-CSF, granulocyte/macrophage-colony stimulating factor; GMF, fusogenic membrane glycoprotein; DC, dendritic cell; hsp, heat shock protein; IL, interleukin; CMV, cytomegalovirus; APC, antigen-presenting cell; RT-PCR, reverse transcription-PCR; VSV-G, vesicular stomatitis virus G glycoprotein; PEG, polyethylene glycol.

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Detection of Cytokine RNA from s.c. Vaccination Sites Using the RT-PCR. Premarked skin samples at the site of vaccine injection were removed from animals at the time periods shown and snap frozen in liquid nitrogen to ensure conservation of the RNA. RNA was prepared by homogenization of the tumor with Qiagen RNA extraction kit, followed by RNA extraction according to the manufacturer’s instructions. RNA concentrations were measured, and 1 µg of total cellular RNA was reverse transcribed in a 20-µl volume using oligo(dT) as a primer and Moloney murine leukemia virus reverse transcriptase (Pharmacia LKB Biotechnology, Milton Keynes, United Kingdom). A cDNA equivalent of 1 ng of RNA was amplified by the PCR using primers specific for individual murine cytokines. PCR was performed by standard techniques.

Transfection of Tumor Cells with the VSV-G FMG to Induce Syncytial Formation. Tumor cells (10⁶) were cultured to 80–90% confluence and transfected with 1–2 µg of pCMV-VSV-G (a kind gift of Dr. Y. Takeuchi, London, United Kingdom) or pCMV-MLV G-E124, a point mutant of VSV-G that abolishes fusion of the protein by >90% (Ref. 50; a kind gift of Dr. M. Whitt, University of Tennessee, Memphis, TN). The pCMV-VSV-G and pCMV-MLV G-E124 plasmids are identical except for the single amino acid change in the VSV-G cDNAs and contain the relevant VSV-G cDNA expressed from a human CMV promoter. Twenty-four h later, cells were incubated in medium of pH 5.7 for 2 min, washed three times in PBS, and returned to normal medium. Twenty-four to 48 h later, cells were extensively fused with up to 80% of tumor nuclei involved in syncytia. Fusing cell vaccines were prepared from such cultures 48 h after pH drop.

DC Culture. DCs were cultured from the bone marrow of C57BL mice according to a protocol modified from that of Inaba et al. (51).

Preparation of Peritoneal Macrophages. Mice were euthanized, the abdominal skin was retracted to expose the peritoneal wall, and 8 ml of DMEM containing 10% FCS was injected into the peritoneal cavity. Approximately 6 ml of fluid were withdrawn, containing resident peritoneal macrophages; the cells were washed and resuspended in medium containing penicillin and streptomycin for further analysis.

In Vivo Studies. All procedures were approved by the Mayo Foundation Institutional Animal Care and Use Committee. C57BL/6 or C3H-deficient nude mice were age- and sex-matched for individual experiments. To establish s.c. tumors, 2 × 10⁶ B16 cells or 2 × 10⁶ M2639 cells were injected s.c. (100 µl) into the flank region. Animals were examined daily until the tumor became palpable, whereafter the diameter, in two dimensions, was measured three times weekly using calipers. Animals were killed when tumor size was approximately 1.0 × 1.0 cm in two perpendicular directions. Animals were considered to have a tumor (to distinguish from a swelling or inflammation in reaction to injection) when a tumor measurement was in excess of 0.2 cm in the longest diameter. All groups of mice in any one individual experiment were rechallenged on the same occasion using the same preparation of cells. The animals were rechallenged 14 days after the last vaccination. A naive group of mice was also injected with these cells at the same time.

Prophylactic Tumor Protection Model. B16 and K1735 vaccines were all prepared from tissue culture-propagated cell lines. Mice received three s.c. vaccinations (100 µl in PBS into the flank region) of 2 × 10⁶ irradiated (100 Gy) parental B16 cells or irradiated parental or gene-modified K1735 cells 7 days apart. Mice were then rechallenged with s.c. injection of 2 × 10⁶ parental B16 cells on the opposite flank as described above. After irradiation, fusion cell vaccines were harvested by trypsinization, washed three times in PBS, and resuspended in 1.2 ml of PBS. Preparation of Peritoneal Macrophages. One hundred-µl injections were administered s.c. as the vaccine. Control experiments using nonfused cells show that each vaccine preparation in this way corresponded to a dose of approximately 2 × 10⁶ cells/vaccine.

Treatment of Established Tumors: “Tumor Therapy.” On day 1, 2 × 10⁶ B16 cells were injected s.c. into the right flank of syngeneic C57BL/6 mice (“primary tumor”). At this dose of cells, 90–100% of animals consistently developed progressively growing tumors that, in the absence of any vaccine treatment, took ~21 days to grow to a size of 1 cm², at which time the animals were sacrificed. When the primary tumor became palpable (typically 3–7 days after injection), 2 × 10⁶ irradiated, parental, or gene-modified cells (“treatment cells”) were injected s.c. on the contralateral flank. This vaccine was repeated twice more at 24-h intervals. The sizes of the primary tumors were measured twice/week until either one reached a size of 1 cm², at which time animals were sacrificed.

RESULTS

K1735 Irradiated Cell Vaccines Are Immunostimulatory at the Vaccine Site. Vaccination with neo-marked, irradiated K1735 cells induced a marked immune infiltrate consisting predominantly of macrophages (Fig. 1, A and B). RT-PCR analysis of the injection site confirmed that the allogeneic cells are cleared by 48–72 h after injection (as seen by disappearance of detectable signal for the neo gene; Fig. 1C). Consistent with the immunohistochemical analysis, we observed a significant proinflammatory cytokine response to the vaccine cells s.c. with the induction of TNF-α and IFN-γ at 24–96 h after vaccination (Fig. 1C). These results suggest that the allogeneic cells produce an immunostimulatory response in vivo. However, K1735 cells modified to express GM-CSF, IL-12, hsp70, or IFN-γ did not enhance the immunizing potential of irradiated K1735 cells (data not shown), consistent with previous findings (28).

K1735 and B16 Cells Share Only a Few Melanoma Antigens. RT-PCR analysis of K1735 and B16 cells showed that of eight melanoma antigens tested, only three were shared (Table 1). In addition, expression of these three shared antigens was consistently lower in K1735 than in B16 cells. These data suggest that a paucity of shared antigens between the cell lines, consistent with the findings of Peter et al. (30), may contribute to the poor vaccine efficacy.

VSV-G-mediated Tumor Cell Fusion Enhances the Efficacy of Autologous/Allogeneic Cell Vaccines. We reasoned that fusing tumor cells to each other through the immune stimulatory mechanisms associated with cell killing through FMG expression may liberate relevant tumor antigens from vaccine cells in an immunologically effective context. The VSV-G is nonfusogenic until the ambient pH is lowered to pH 5.5–5.7, which induces a conformational change that converts the protein into a fusogenic conformation (50, 54). Because the VSV-G envelope protein may be a potent adjuvant of itself (31, 37–39), we also used a mutant VSV-G, G-E124, in which a single amino acid mutation reduces the amount of cell fusion induced by expression of the gene by >90% (50). Transfection with this mutant VSV-G also served as a control for the transient pH drop to which all
fusing vaccine preparations were exposed. Transfection of tumor cells with VSV-G, followed 24 h later by a 2-min drop in pH to 5.7, caused fusion of cells such that between 60 and 90% of the cells in vitro were involved in syncytia 48 h after the pH drop (Fig. 2C). Transfection with VSV-G G-E124 routinely fused minimal numbers of cells (Fig. 2B; Ref. 50).

We tested the effects of vaccination of C57/BL mice with VSV-G-mediated fusing cell vaccines in a prophylactic protection model. Fusion of B16 cells to each other was reproducibly, but only moderately, a more effective vaccine than either B16 transfected with the nonfusing VSV-G or irradiated B16 cells (Fig. 3A). Fused K1735 cells similarly generated moderate, therapeutic benefit relative to K1735 cells transfected with the fusion-mutant VSV-G G-E124 (Fig. 3B).

However, the fusion of a 1:1 mixture of K1735:B16 cells with VSV-G generated a very effective vaccine with up to 80% of mice surviving rechallenge long term (>60 days), depending upon the individual experiment (Fig. 3C). The range of protection in the experiments we have carried out with the 1735/B16-FUS vaccine was from 30 to 80% of the animals in the treatment groups. Overall, the experiment was carried out seven times, and the protection seen was 7 of 10, 8 of 10, 3 of 10, 7 of 10, 7 of 10, 8 of 10, and 7 of 10 mice protected long term against the rechallenge of parental B16 cells. In

Table 1  Melanoma-associated antigens shared between B16 and K1735 cells

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Each case, 100% (10 of 10) animals vaccinated with irradiated B16 cells, as negative controls, developed tumors. These effects were attributable neither solely to the adjuvant effect of the VSV-G immunogen nor to vector-related components, nor to the brief exposure of cells to low pH, because the fusion defective mutant VSV-G G-E124 gave only minimal therapeutic gains, although the expressed protein reaches the surface of the cells and differs antigenically only in a single amino acid (50).

The mechanism of fusion and the allogeneic fusion partner are critical for vaccination efficacy. The prophylactic vaccination experiments of Fig. 3 were also carried out by fusing the K1735:B16 cells using PEG (55). PEG-mediated fusion of tumor cells gave no delay in the appearance of tumors on rechallenge (Fig. 4). Similarly, VSV-G-mediated fusion of B16 cells with allogeneic fibroblasts (NIH3T3) was ineffective at generating tumor protection (Fig. 4). Therefore, the K1735 fusion partner presumably enhances the vaccination through donating melanoma-associated antigens and/or other, as yet undefined, immunological activation signals.

Fusing tumor cell vaccines are effective in a therapy model of disease. We also tested the fusing tumor cell vaccines in a model of established disease (46). Fusing B16:K1735 vaccines cured up to 80% of mice with established disease in this model (Fig. 5A). Both K1735:K1735 and B16:B16 fusing vaccines consistently produced a significant delay in growth of the tumor and, depending on the experiment, cured between 20 and 40% of the mice (Fig. 5A). Other vaccines produced no therapeutic effect (Fig. 5A).

The long-term vaccinating capability of fusing tumor cell vaccines is both tumor specific and T-cell dependent. In the experiment shown in Fig. 5A, we rechallenged the eight mice that had been cured of their tumors by treatment with K1735:B16 fusing tumor vaccines. Of the four mice rechallenged with CMT93 tumor cells, an unrelated colorectal tumor, all four developed tumors (Fig. 5B). In contrast, only one of four mice rechallenged with parental B16 cells developed a tumor (Fig. 5B), showing that fusing vaccines generate long-term, tumor-specific immunity. We also repeated the vaccination protocol of Fig. 3 in athymic nude mice. Fig. 6 shows that the vaccinating ability of the fusing cell vaccines was completely eradicated in animals lacking functional T cells.

Macrophages are activated by syncytial death in vitro. We investigated the response of both macrophages and DCs to fusing tumor cell vaccines. Coculture of labeled macrophages (Fig. 7A) with labeled tumor cells (Fig. 7Ai) showed that macrophages can take up tumor-derived material to a small degree from actively growing cultures (seen as a right shift of staining of the macrophages in Fig. 7Aii), however, when autologous tumor cells were killed through syncytial formation (as described in Refs. 31–33), uptake of labeled material by the macrophages was sometimes enhanced (Fig. 7Av). However, uptake of tumor-derived material was consistently optimal when the target cells contained allogeneic cells (K1735 alone or K1735+B16; Fig. 7Av). No secretion of either IFN-γ or IL-10 could be detected from macrophages cocultured with syncytia. TNF-α was produced at low levels by macrophages in response to allogeneic cells in the absence of cell fusion (Fig. 7Aii) but not to autologous B16 cells alone (data not shown). Fusion of B16 cells activated TNF-α production by macrophages (Fig. 7Av). However, TNF-α production was consistently seen at highest levels in response to fusing cells containing allogeneic cells (Fig. 7Av). Similar experiments with immature murine DCs showed enhanced levels of uptake of tumor material from fusing cells compared with normal cultured cells, but there was no distinction between fusing cell mixes that contained only autologous or allogeneic components (Fig. 7B). Similarly, incubation of immature DCs with syncytia did not induce TNF, IL-10, or IFN-γ secretion.

Syncytial cell killing leads to cross-presentation by DCs of a model tumor antigen from both autologous and allogeneic tumor cells. T cells from OT1 transgenic mice recognize the SIINFEKL epitope of the ovalbumin antigen in the context of H2b (52). DCs loaded with SIINFEKL peptide are recognized by OT1 cells, leading to IFN-γ release (Fig. 8A). B16ova-transfected cells cannot present the SIINFEKL epitope to OT1 cells, either from exogenous loading of the peptide or from endogenous expression of ova (Fig. 8A; unless pretreated with IFN-γ to up-regulate class I MHC
expression, data not shown). Similarly, B16ova incubated with DCs alone do not release ova epitopes for presentation (Fig. 8A). Lysates of B16ova cells killed by freeze thawing or osmotic shock were unable to load DCs with the appropriate epitope of ova for cross-presentation to OT1 cells (data not shown). In one of three experiments, B16ova cells killed by irradiation (100 Gy) were able to serve as a source of ova antigen for DCs in a cross-presentation assay (Fig. 8B). However, B16ova cells fused by VSV-G were a reproducible and potent source by which the ova epitope could be supplied to DCs for subsequent presentation to OT1 cells, although DCs were absolutely required (Fig. 8B). We were unable to generate a K1735 cell line expressing ova but did generate NIH3T3 cells expressing ova as an allogeneic source of the protein. (NIH3T3 cells were unable to present ova to OT1 cells, even with IFN-γ stimulation because of a lack of H2b MHC, data not shown). To a lesser degree than with fusing B16ova cells, NIH3T3ova cells expressing fusogenic VSV-G loaded H2b-positive DCs with ova that led to presentation of the SIINFEKL epitope to OT1 cells (Fig. 8C). Irradiation (Fig. 8C), freeze thaw, or osmotic shock (data not shown) of the allogeneic ova-expressing line was ineffective at promoting cross-presentation of the ova antigen by H2b DCs.

B16 cells (10 mice/group). Under the experimental vaccination conditions used in these experiments, neither irradiated B16 cells nor irradiated K1735 cells generated any protection against B16 challenge relative to naive mice or relative to tumor cells transfected with the fusion defective VSV-G. For clarity, the separate groups of a single experiment are shown. A, VSV-G-transfected, fusing B16 vaccines (B16-FUS) showed reproducible improvements over irradiated B16 cells (not shown) and B16 cells transfected with the fusion defective VSV-G-G-E124 (B16-dFus). B, VSV-G-transfected, fusing K1735 vaccines (K1735-FUS) were consistently superior to K1735 cells transfected with the fusion-defective VSV-G-G-E124 (K1735-dFus), but the gains were in slowing tumor growth rather than survival. C, a 1:1 mixture of K1735 and B16 cells transfected with VSV-G and acid shocked (K1735/B16-FUS) generated long-term protection from rechallenge in 7 of 10 mice in the experiment shown, which was never seen by expression of the fusion-defective VSV-G (K1735/B16-dFus; P < 0.0003). These results are representative of seven different experiments.

Fig. 3. Fusing cell vaccines are potent immunogens in a prophylactic protection model. C57/BL mice were vaccinated with irradiated cells as described for prophylactic protection against rechallenge with parental B16 cells. The percentage of mice developing actively growing tumors (>0.2 cm in diameter) are shown after challenge with parental

Fig. 4. Both the mechanism of fusion and the allogeneic fusion partner are critical for vaccination efficacy. B16 cells were fused either with 1735 cells by VSV-G transfection (1735/B16-FUS) or by PEG (1735-B16-PEG). Alternatively, B16 cells were fused with NIH3T3 cells instead of K1735 using either VSV-G transfection and acid shock (NIH3T3/B16-FUS) or the fusion-defective VSV-G (NIH3T3/B16-dFus). These cell mixtures were used as irradiated cell vaccines in prophylactic vaccination against rechallenge with parental B16 cells. The percentages of mice developing actively growing tumors (>0.2 cm in diameter) are shown after challenge with parental B16 cells (10 mice/group). The 1735/B16-FUS vaccine was significantly more effective than the 1735-B16-PEG (P < 0.0001) vaccine. These results are representative of three separate experiments.
Our results show that an irradiated mixture of autologous and allogeneic tumor cells fused by VSV-G was extremely effective at protecting mice from a subsequent rechallenge with B16 cells. In addition, a similar vaccine was also very potent at curing small, established disease. The immunological mechanisms involved in the generation of short-term therapy may be different from those involved in long-term protection (35, 36, 40, 46, 56–61). In the experiments reported here, a single gene modification, VSV-G-induced tumor cell fusion, is effective in generating both rejection of established tumors as well as long-term, T-cell-mediated protection responses. Syncytia of B16 and K1735 cells are likely to be good targets for natural killer and other nonspecific immune effector killing mechanisms through expression of the viral immunogen VSV-G (37–39). In addition, direct syncytial-mediated cell killing will also release immune-stimulatory molecules at the vaccine site, which will recruit and activate host APCs (62), and stress proteins expressed within the syncytia (31) should contribute directly to immune stimulation (35, 36, 40, 57). Thus, released tumor-associated antigens will then become available for cross-presentation by host APCs at the site (58–61). Significantly, PEG-mediated cell fusion, characterized by small, disorganized clumping aggregates rather than the large, organized syncytial structures that are characteristic of VSV-G fusion, was ineffective in generating potent vaccines. Thus, the activation of cellular stress programs in response to VSV-G-mediated fusion (31, 63) may mimic a pathological type situation, which is sensed by the cell as an immunologically relevant situation (41, 43).

Our work described here builds on a variety of previous approaches that have investigated the use of allogeneic/autologous vaccine preparations. For example, Staib et al. (64) demonstrated that a mixture of autologous B16-derived melanoma cells with allogeneic Cloudman-S91 melanoma cells was able to vaccinate mice against growth of intracerebral B16 metastases. In addition, several immunizing semiallogeneic (65–69) or even semi-xenogeneic (70, 71) hybrid cells have also been described. Most of these probably act in a similar way to those hybrids formed between tumor cells and professional APCs (7, 55, 72) by enhancing direct presentation of tumor-associated antigens. This approach has shown great promise in recent clinical protocols (7). However, neither the K1735 nor B16 cells are well equipped for antigen presentation. The protective immunity provided by fused autologous cells alone (B16) or fusing allogeneic (1735) vaccines was similar (Fig. 3, A and B). This may indicate that 1735 cells do indeed possess similar levels of relevant tumor antigens as B16 cells and that the improved efficacy of K1735 cells fused with B16 cells that we observed in the experiments of Figs. 3C and 4 may be attributable to

**DISCUSSION**

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a mechanism entirely different from simple transfer of relevant antigens. Indeed, the results of Fig. 8 suggest that fusion mediates the release of available antigens in a form that promotes uptake and cross-presentation by DCs. Alternatively, it may be that the adjuvant effects of allogenicity compensate for low levels of antigens in the 1735 vaccine (as suggested by Table 1 and Ref. 30) such that the combination of allogenicity plus the effects of fusion equalize the efficacy of the autologous and allogeneic vaccines. The failure of vaccines with NIH3T3 cells as the fusion partner suggests, however, that the allogeneic melanoma cell line also provides additional important signals to the vaccine preparation. These could be relevant antigens and/or some particularly important immune-stimulatory molecules that are not provided by the allogeneic fibroblasts (73).

Taken together, the data reported here also show that macrophages recruited to the s.c. site of allogeneic cell vaccination (as seen in Fig. 1) will be significantly more activated in situ by the presence of fusing cells, permitting initiation of a more effective antitumor immune response (40). Importantly, we also show that syncytial cell death, but not other forms of tumor killing, promotes cross-presentation of tumor-associated antigens by immature DCs. In this respect, it may be that stress proteins, such as hsp, serve in some way as potentiators for uptake of antigens into the DCs (74–76). For example, Tamura et al. (77) demonstrated that hsp preparations derived from autologous cancer cells could treat local and metastatic disease in a range of murine tumor types. Other routes for uptake of antigen from dying cells and subsequent cross-presentation by DCs have also been reported that rely upon levels and routes of antigen release (21, 22, 78–80).

Clinically, we believe that these data are very significant for the design of tumor cell vaccines. By modifying either patient-recovered tumor explants or established allogeneic cell lines with a gene for an FMG, fusing tumor cell vaccines can be produced in vitro for patient use. An important pragmatic issue for design of clinically useful vaccines is whether it is more important to use allogeneic cells as the principal vaccine component and fuse these with syngeneic cells or vice versa. To address this issue, we are currently comparing the efficacy of vaccines consisting of MHC loss variants of B16 cells, combined with transfection of single syngeneic or allogeneic MHC molecules. Preliminary results suggest that the presence of allogeneic cells of the same histological type (as opposed to that of a different histological type, as seen in Fig. 4 where the fusion partner was allogeneic but not melanoma) is the predominant
critical parameter for maximal vaccine efficacy. Thus, our data suggest that an allogeneic cell vaccine engineered to undergo F MG-mediated fusion could be boosted by the addition of patient tumor cells prepared over a very short period of time after surgery (81) to obtain the allogeneic: autologous mix that was effective in our studies described here.

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