Critical Components of a DNA Fusion Vaccine Able to Induce Protective Cytotoxic T Cells Against a Single Epitope of a Tumor Antigen

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DNA vaccines can activate immunity against tumor Ags expressed as MHC class I-associated peptides. However, priming of CD8+ CTL against weak tumor Ags may require adjuvant molecules. We have used a pathogen-derived sequence from tetanus toxin (fragment C (FrC)) fused to tumor Ag sequences to promote Ab and CD4+ T cell responses. For induction of CD8+ T cell responses, the FrC sequence has been engineered to remove potentially competitive MHC class I-binding epitopes and to improve presentation of tumor epitopes. The colon carcinoma CT26 expresses an endogenous retroviral gene product, gp70, containing a known H2-Ld-restricted epitope (AH1). A DNA vaccine encoding gp70 alone was a poor inducer of CTL, and performance was not significantly improved by fusion of full-length FrC. However, use of a minimized domain of FrC, with the AH1 sequence fused to the 3’ position, led to rapid induction of high levels of CTL. IFN-γ-producing epitope-specific CTL were detectable ex vivo and these killed CT26 targets in vitro. The single epitope vaccine was more effective than GM-CSF-transfected CT26 tumor cells in inducing an AH1-specific CTL response and equally effective in providing protection against tumor challenge. Levels of AH1-specific CTL in vivo were increased following injection of tumor cells, and CTL expanded in vitro were able to kill CT26 cells in tumor bearers. Pre-existing immunity to tetanus toxoid had no effect on the induction of AH1-specific CTL. These data demonstrate the power of epitope-specific CTL against tumor cells and illustrate a strategy for priming immunity via a dual component DNA vaccine. The Journal of Immunology, 2002, 169: 3908–3913.

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eoxyribonucleic acid vaccines offer a novel strategy for inducing preventative immune responses against infectious organisms (1, 2). Vaccination against cancer is more demanding, since priming has to take place in a setting where tolerance and immune deficiency may be present. A further difficulty is that tumor Ags are generally of low immunogenicity. However, the relative ease of manipulation of DNA vaccine constructs, combined with the range of preclinical models available for testing, is providing potential solutions to these obstacles.

One problem for rational design is that the mechanism by which injected DNA leads to generation of immunity is not yet fully understood. Although it is clear that CpG motifs in the bacterial plasmid backbone activate the mammalian innate immune system, with a consequent production of cytokines, including IL-12 and IFN-γ (3–5), the fate of the encoded protein is less clear. Intramuscular injection generates a depot of Ag that is delivered to APC, presumed to be mainly dendritic cells (DC) (6, 7, 8). However, the mode of transfer to DC depends on the nature of the Ag and on the cellular location within the muscle cell (8, 9). Injection into the skin by gene gun is more likely to directly transfect DC, and on the cellular location within the muscle cell (8, 9). Injection into the skin by gene gun is more likely to directly transfect DC, although even in this situation, keratinocytes may provide a major source of Ag (10, 11). One important component of the transfer process is likely to be heat shock proteins which are located in the cytosol and endoplasmic reticulum and can act as cargoes for transfer of peptides to receptor-bearing DC (12, 13).

The concept that weak tumor Ags could be rendered more immunogenic by fusion to foreign molecules was already clear from protein Ags, where linkage to keyhole limpet hemocyanin is a common strategy. Anti-tumor Ab levels are increased due to provision of high levels of keyhole limpet hemocyanin-specific CD4+ T cell help. A similar concept appears to operate for DNA vaccines where we have used a fused pathogen-derived sequence to amplify Ab responses against idiotypic Ag from B cell tumors (14, 15). In our case, the fragment C (FrC) component of tetanus toxoid (TT), fused to the 3’ position of single-chain Fv, promoted anti-idiotypic Ab able to protect against lymphoma (15) and is now in clinical trial, with encouraging results emerging. Similar promotional strategies for idiotypic Ags have been reported using xenogeneic Ig sequences (16) and a sequence encoding a plant viral coat protein (17). For DNA vaccines encoding single-chain Fv-FrC, fusion was an absolute requirement, consistent with activation of cognate T cell help (14, 15). Interestingly, the same fusion design was effective in amplifying a cellular response against idiotypic Ag secreted by a myeloma model (15, 17). In this case, protection was apparently mediated by CD4+ T cells, presumably acting via an indirect mechanism, as described using transgenic CD4+ T cells in the MOPC315 plasmacytoma (18, 19).

The challenge for tumor vaccines aimed at the wide range of emerging tumor Ags is to design a strategy to induce CD8+ CTL responses. For DNA vaccines, we made two changes to the FrC fusion design. The first was to remove the second domain of FrC which includes several mouse and human MHC class-I binding motifs (20–22), thereby decreasing the possibility of competition with tumor-derived epitopes during the establishment of immunodominance by responding CTL (22–24). We retained the first domain which contains a leader sequence and a promiscuous MHC

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Abbreviations used in this paper: DC, dendritic cell; FrC, fragment C; TT, tetanus toxoid.
class-II binding peptide, p30 (25). We fused a sequence encoding a candidate target epitope from the second domain of FrC to the 3′ position (22). The combination of the first domain of FrC with the C-terminal peptide led to high levels of CTL able to protect against a transfected target cell.

In the present study, we have applied this design to a known endogenous tumor Ag derived from a retrovirus (26). We have demonstrated the efficacy of the design for a colon carcinoma and have been able to detect specific CTL ex vivo. Comparison with a cellular vaccine (CT26 tumor cells engineered to secrete GM-CSF) showed that CTL induced by a DNA vaccine, and specific for only a single epitope, have equivalent power to disperse of aggressive tumor cells. Interestingly, pre-existing Ab against FrC had no effect on the induction of CTL by the fusion gene.

Materials and Methods

Cells

The P815 mastocytoma and the CT26 colon carcinoma cell lines were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Life Technologies, Paisley, U.K.), 1 mM sodium pyruvate, 2 mM l-glutamine, nonessential amino acids (1% of 100× stock), 25 mM HEPES buffer, and 50 mM 2-ME. CT26 cells that had been genetically modified to secrete GM-CSF (CT26/GM-CSF) were a kind gift from Dr. D. Pardoll (Johns Hopkins University, Baltimore, MD). CT26 cells were harvested by incubating with Ca/Mg-free medium (Hanks’ MEM, modified; Sigma-Aldrich, St. Louis, MO), containing 0.2 mM EDTA, for 10 min before washing with PBS.

Peptides

The H2-k restricted gp70 CTL epitope (AH1) sequence (SPSYVVYHQF) has been described previously (26). It was synthesized commercially and supplied at >95% purity (Peptide Protein Research, Southampton, U.K.). The H2-k restricted CTL epitope from TT (TT1228-1236: GYNAPGIP), located in the C-terminal domain of FrC (21), was synthesized in-house on a Shimadzu PMS peptide synthesizer (Shimadzu Scientific Instruments, Columbia, MD), using Fmoc chemistry, and checked for purity by HPLC.

Construction of DNA vaccines

The structures of the DNA vaccines are indicated in Fig. 1. Construction of the DNA vaccine containing the gene encoding the full-length, two-domain sequence of FrC (p.FrC, aa 865-1316 of TT (TT865-1316)), with a leader sequence spanning the region of the gp70 protein that encodes the AH1 CTL epitope (SPSYVVYHQF).

Using these constructs as templates (p.FrC, p.DOM, and p.env), another three DNA vaccines were constructed: 1) DNA encoding the first domain of FrC, with DNA encoding the AH1 CTL epitope fused to the carboxyl terminus (p.DOM-AH1); 2) DNA encoding two-domain FrC with sequence encoding the first domain of FrC (p.FrC70) (which fuses AH1-encoding sequence of p.DOM-AH1 and primer R1. Third, these two gel-purified PCR products were combined and assembled by PCR-SOEing using primers F1 and R1. The assembled vaccine PCR product was ligated into pcDNA3 using HindIII and NotI restriction sites.

The gp70 vaccine was also constructed using a three-step procedure: first, the BCL1 leader sequence was amplified using the primers F1 and 5′-GGAGATTCACTGGTCAACCTGTTACAGGTTGATAAGCTCC-3′, which contains a gp70 overlapping sequence. Second, using p.env as a template, the gp70 sequence was amplified using the primers 5′-ACAGCTTGAATCTCAGACG-3′ and 5′-AAACGCGCCCGTATATACGATTCTGTAA-3′ (primer R1). Third, these two gel-purified PCR products were combined and assembled by PCR-SOEing using primers F1 and R1. The assembled vaccine PCR product was ligated into pcDNA3 using HindIII and NotI restriction sites.

All constructs encode the BCL1 leader sequence at the amino terminus. Integrity of all constructs was confirmed by DNA sequencing.

Vaccination protocol and CTL assay

BALB/c mice, bred in-house, were vaccinated at 6–10 wk of age with a total of 50 μg of DNA in normal saline, injected into two sites in the quadriceps muscles on day 0, or 1 × 10⁶ irradiated (5000 rad) CT26/GM-CSF cells by the s.c. route into the left flank. To assess the effect of tumor challenge, mice were vaccinated with DNA as indicated and then injected s.c. with 1 × 10⁶ CT26 tumor cells into the right flank on day 14 before culling and ex vivo FACS analysis on day 20. To establish CTL lines, mice were sacrificed at day 14. Splenocytes were pooled from vaccinated mice and single-cell suspensions were prepared in supplemented RPMI 1640 medium, as described above. Splenocytes were resuspended in 40 ml of medium, at 3 × 10⁵ cells/ml, and added to 80-cm² flasks along with recombinant human IL-2 (20 U/ml; PerkinElmer, Foster City, CA) and AH1 peptide (10 μM).

Cytolytic activity of the T cell cultures was assessed by standard 4- to 5-h³¹Cr release assays following 6 days of stimulation in vitro, as previously described (22). Targets included P815 or CT26 cells, either alone or incubated with test (AH1) or control peptide (TT1228-1236: GYNAPGIP) (21). The specific lysis was calculated by the standard formula: [(release by CTL alone) - (release by test peptide alone)] / (release by test peptide alone) × 100.

Animal welfare and experimentation was conducted in accordance with the local Ethical Committee and the U.K. Coordinating Committee for Cancer Research, (London, U.K.) guidelines, under Home Office license.

Ex vivo intracelluar IFN-γ assay

Viable cells were selected by density centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway). Cells were incubated for 4 h at 37°C in 96 U-well plates, at 5 × 10⁵ cells/well, along with 10 U/well rIL-2, 1 μM peptide, and 1μl/well Golgi plug. Samples were blocked with 2% complement-free mouse serum (1 min, 4°C) before labeling with 1μg/well APC anti-mouse CD8b.2 (Ly-3.2, clone 53-5.8), and FITC anti-mouse MHC class II I-A/E (E29.9), or isotype controls (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixed with 1% formaldehyde (20 min, 4°C). Following surface labeling, cells were fixe...
Tumor therapy in vivo by adoptive transfer

Mice were challenged by s.c. injection of $1 \times 10^5$ CT26 tumor cells into the right flank. Two days later, $6.5 \times 10^6$ AH1-specific CTL were adoptively transferred into tumor-bearing animals by i.v. injection (CTL were expanded by two rounds of in vitro stimulation of splenocytes from mice that had been vaccinated with p.DOM-AH1 14 days earlier, as described above). Mice also received 90,000 U of human rIL-2 i.p., on the day of CTL transfer and for the subsequent 3 days. Mice were sacrificed when the resulting tumor reached 1.5 cm in mean diameter and the day of death was recorded.

Prevaccination studies

Mice were prevaccinated at 6–10 wk of age with 8 IU of adsorbed tetanus vaccine (Pasteur Merieux, Lyon, France) on alum, injected into two sites in the quadriceps muscles. Control animals were given either alum alone or saline. On day 21 or day 42 posttreatment, mice were vaccinated with 50 μg of DNA in normal saline injected into two sites in the quadriceps muscles. Mice were sacrificed 2 wk later and spleens were harvested for ex vivo FACS analysis. Lytic activity of CTL bulk cultures was assessed 6 days later as described above.

Results

Induction of IFN-γ-producing CD8$^+$ T cells by DNA vaccines encoding gp70 sequences

DNA vaccines encoding sequences derived from gp70, all including the murine leukemia virus epitope (SPSYVYHQF: AH1) or controls (Fig. 1) were tested for their ability to induce AH1-specific CD8$^+$ T cell responses following a single i.m. injection. At day 14, splenocytes were tested for peptide-specific IFN-γ-producing CD8$^+$ T cells immediately ex vivo. Results in Fig. 2 are representative of multiple experiments showing that the minimized DNA vaccine, containing a single domain of FrC fused to the AH1-encoding sequence (p.DOM-AH1) (Fig. 1), was able to induce detectable specific IFN-γ-positive CD8$^+$ T cell responses. In Fig. 2, 1.3% of the CD8$^+$ T cells were positive as compared with 0.12% in the p.DOM control. The p.gp70 construct failed to induce a significant response (0.08%). However, fusion to the full-length FrC adjuvant sequence (p.FrC-gp70) did promote a response (1.0%), although this was consistently less than that induced by p.DOM-AH1. The ability of the DNA vaccines to induce a response was compared with CT26 cells transfected with GM-CSF, a known cellular vaccine able to induce protective immunity against CT26 tumor, with AH1 identified as an immunodominant peptide (26). At day 14, this vaccine was able to induce AH1-

responding T cells (0.40%) but was reproducibly less efficient than the p.DOM-AH1 vaccine (Fig. 2).

Cytotoxic activity of induced CD8$^+$ T cells

The ability of the IFN-γ-producing CD8$^+$ T cells to kill target cells was then tested following a 6-day expansion with peptide-loaded APC in vitro. Although the CD8$^+$ T cells induced by p.DOM-AH1 were able to kill peptide-loaded target cells efficiently and reproducibly (Fig. 3a), none of the other constructs led to effective cytotoxicity. Although the p.FrC-gp70 construct did induce IFN-γ-producing CD8$^+$ T cells (Fig. 2), these did not expand reproducibly in vitro and failed to generate cytotoxic T cells. In contrast, if the restimulation was conducted using APC loaded with a MHC class I-binding peptide from FrC (GYNAPGPIPL), CTL able to kill FrC peptide-loaded target cells were detectable (data not shown). The p.FrC-gp70 construct therefore is capable of generating CD8$^+$ responses against both components of the fusion protein, but those against AH1 appear weak. Even following three rounds of i.m. injections of p.FrC-gp70 or p.gp70 on days 0, 21, and 42, no significant CTL activity against AH1 peptide could be expanded, whereas high levels of CTL were induced by p.DOM-AH1 using this protocol (data not shown).

Specific CTL induced by p.DOM-AH1 were able to kill untreated CT26 target cells in vitro with high efficiency (Fig. 3b). CT26 cells transfected with GM-CSF also induced CTL able to kill both peptide-loaded P815 cells and untreated CT26 cells (Fig. 3b), but levels were reproducibly lower than those induced by the p.DOM-AH1 vaccine. We occasionally observed elevated background lysis when using P815 as a surrogate target cell for AH1-specific CTL (Fig. 3b), possibly due to low level expression of gp70 by this cell line (26, 27). Lysis of gp70-negative cell lines, such as the murine plasmacytoma MOPC315, was always negligible (data not shown).

Effect of tumor challenge on levels of AH1-specific CD8$^+$ T cells

Mice vaccinated with p.DOM-AH1 or control p.DOM were challenged with a s.c. injection of CT26 cells and levels of AH1-specific IFN-γ-producing CD8$^+$ T cells were measured directly ex vivo 6 days later. As shown in Fig. 4, there was a clear increase in the level of AH1-specific CD8$^+$ T cells.
peptide for 6 days in vitro before measuring CTL activity by a 51 Cr release panel as indicated. At day 14, splenocytes were restimulated with AH1 cells was observed by AH1-specific CTL (20%). The SEM for each data point was low (<1% lysis). Lysis of P815 cells loaded with control peptide was negligible in each case (<5%), although some lysis of gp70-positive P815 cells was observed by AH1-specific CTL (<20%). The SEM for each data point was low (<1% lysis). Representative data are shown; similar results were obtained in three of three experiments.

FIGURE 3. a. Repositioning the AH1 epitope from the embedded site in gp70 to the C terminus of the first domain of FrC amplifies the specific anti-AH1 CTL response. Mice were vaccinated with p.FrC (●), p.DOM (□), p.FrC-gp70 (▲), p.gp70 (●), or p.DOM-AH1 (■). At day 14, splenocytes were restimulated with AH1 peptide for 6 days in vitro before measuring CTL activity by a 51 Cr release assay using P815 target cells loaded with AH1 peptide. Lysis of P815 cells loaded with control peptide was negligible in each case (<5%), although some lysis of gp70-positive P815 cells was observed by AH1-specific CTL (<20%). The SEM for each data point was low (<1% lysis). Representative data are shown; similar results were obtained in three of three experiments. b. Improved efficacy of the modified p.DOM epitope fusion vaccine (p.DOM-AH1) over the CT26/GM-CSF cellular vaccine to induce AH1-specific CTL capable of killing CT26 tumor cells in vitro. Mice were vaccinated with either the p.DOM-AH1 DNA vaccine (left panel) or irradiated CT26/GM-CSF cells (1 × 10⁶ s.c.; right panel) as indicated. At day 14, splenocytes were restimulated with AH1 peptide for 6 days in vitro before measuring CTL activity by a 51 Cr release assay using P815 cells pulsed with AH1 peptide (●), P815 cells alone (■), or CT26 tumor cells (▲). Background levels of CT26 lysis following vaccination with the control vaccine were <20% (data not shown). The SEM for each data point was low (<3.3% lysis). Representative data are shown; similar results were obtained in three of three experiments.

CTL induced by p.DOM-AH1 vaccination protect against CT26 cell growth in vivo

The DNA vaccines containing the AH1 sequence were tested for their ability to induce protective immunity against s.c. CT26 challenge, and performance was compared with the known protective efficiency of the transfected cellular vaccine. Results (Fig. 5) show that the p.DOM-AH1 construct, containing only a single epitope from CT26, generates highly effective protective immunity against tumor. Protection, which was mediated entirely by CD8⁺ T cells (depletion data not shown), was at least as effective as the cellular vaccine. No significant protection was induced by plasmids containing the gp70 sequence alone (p.gp70) or fused to full-length FrC (p.FrC-gp70). Therefore, there was a clear correlation between the ability to induce cytolytic T cells and protection.

Epitope-specific CTL induced by the p.DOM-AH1 vaccine were expanded in vitro and tested for their ability to protect mice that had already been challenged with CT26 tumor. Transfer of 6.5 × 10⁶ cells protected mice effectively against tumor growth (Fig. 6), showing that the CTL could invade the s.c. site in vivo and kill tumor cells in situ.

FIGURE 4. CT26 tumor challenge boosts the levels of AH1-specific CTL in mice that have been primed with the modified p.DOM epitope fusion vaccine (p.DOM-AH1). Mice were vaccinated with p.DOM or p.DOM-AH1 and challenged 14 days later by s.c. injection of 1 × 10⁶ CT26 tumor cells as indicated. At day 20, mice were culled and ex vivo FACs analysis was used to measure the percentages of CD8⁺ T cells containing intracellular IFN-γ. Representative data are shown; similar results were obtained in three of three experiments.

FIGURE 5. Comparison of the abilities of DNA vaccines containing embedded or repositioned peptides to induce protective immunity against challenge with CT26 tumor cells. Mice were vaccinated with p.FrC (△), p.DOM (○), p.FrC-gp70 (▲), p.gp70 (●), or p.DOM-AH1 (◇) DNA vaccines on days 0 and 21 or irradiated CT26/GM-CSF cells (1 × 10⁶ s.c.; ■) on day 14. Control mice (□) received no vaccine. At day 28, 1 × 10⁵ CT26 tumor cells were injected s.c. and mice were sacrificed when tumor size reached 1.5 cm in diameter. Data from one of two experiments with similar results are shown. Both p.DOM-AH1 and CT26/GM-CSF induced significant protection (p < 0.0022, χ² log rank test) compared with all other groups.
The SEM for each data point was low (\( \chi^2 \))). Mice were received either naive splenocytes (\( \mathbf{\bullet} \)) or no cell transfer (\( \mathbf{\blacktriangle} \)). Control animals had been vaccinated with p.DOM-AH1 14 days earlier). Control animals expanded by two rounds of in vitro stimulation of splenocytes from mice that had been vaccinated with p.DOM-AH1 14 days earlier). Control animals were sacrificed when tumor size reached 1.5 cm in diameter. Transfer of AH1-specific CTL induced a significant cure from tumor (\( p < 0.0001, \chi^2 \) log rank test) compared with all other groups. Data from one of two experiments with similar results are shown.

Effect of pre-existing immunity against tetanus toxoid on induction of specific CTL

To apply this approach to the clinic, it was important to assess any effect of pre-existing immunity against tetanus toxoid on the response to a vaccine containing tetanus toxoid-derived FrC as an adjuvant. Mice were vaccinated with tetanus toxoid alum at day \(-21 \) before being vaccinated with p.DOM-AH1 or p.DOM control on day 0. High levels of anti-FrC Abs were detected at day 0 in both groups (Fig. 7) (~12,000 U/ml at day \(-42 \) and ~3000 U/ml at day \(-21 \)). Following DNA vaccination, AH1 epitope-specific CTL were measured ex vivo on day 14. There was no detectable difference between the numbers of responding CTL in mice preimmune to tetanus toxoid at either time point or controls (Fig. 7a). There was also no effect on the cytotoxic activity of the induced cells following a 6-day stimulation in vitro (Fig. 7b). It appears therefore that anti-FrC Abs do not influence the induction of CTL following i.m. injection of FrC-encoding vaccines.

Discussion

The goal of vaccination against cancer is elimination of tumor cells, and CTL are powerful mediators of attack. However, the intensity of the immune response appears to determine tumor clearance (28) and therefore high levels of CTL will be required. The CT26 tumor presents a useful model for testing vaccine efficiency, since it expresses the endogenous murine leukemia virus \( \text{env} \) gene as a tumor-associated Ag, gp70 (26). This Ag is present in several tumors of BALB/c and C57BL/6 mice and carries an immunodominant H2-Ld-restricted epitope, AH1, which is a target for CTL attack (26). Vaccination with irradiated CT26 cells transfected with GM-CSF induced CTL able to recognize AH1 and to protect against tumor challenge (26).

For rationally designed vaccines, especially those using gene-based delivery, it is desirable to focus on specific immunogenic sequences in tumors. The AH1 peptide is known to be only moderately immunogenic (29), and, although gp70 has not been detected in normal tissues (26), it has been suggested that this is due to natural expression which may tolerate high-affinity T cells. Amino acid substitutions in the peptide which stabilizes MHC-peptide-TCR complexes increased the ability of preloaded DC to induce CTL responses, but protection against tumor challenge remained modest (29).

Delivery of AH1 via recombinant vaccinia virus either from full-length gp70 or from a minigenic with a leader sequence also failed to protect against challenge with CT26 tumor (29). However, a similar vaccinia virus delivery of the full-length gp70 sequence was able to induce T cell responses against AH1-epitope-pulsed splenocytes after expansion in vitro (30). This response was sufficient to suppress metastases in the lung, a site where low avidity T cells can be effective due to small tumors and massive in situ inflammatory conditions (31). Inhibition of established lung metastases was also achieved using peptide-loaded DC pretreated with CD40 ligand (31). These data all indicate that AH1 peptide can be immunogenic but only if combined with maximal costimulatory factors.

DNA vaccines allow simple delivery of the gp70 sequence, and the effects of adding promotional sequences to amplify and direct immune outcome can be assessed. For gp70, the effect of fusing the \( \text{lacZ} \) gene from \( \text{Escherichia coli} \) to the 594-bp sequence of gp70 was investigated (27). Although this fusion gene was able to induce high levels of anti-gp70 Ab, only modest levels of CTL able to kill CT26 cells in vitro were detected. Protection against tumor challenge was also evident, but was quite weak. The observation that fusing a large sequence to a tumor Ag sequence to provide additional T cell help for Ab induction reflects our strategy for raising anti-idiotypic Abs to...
attack B cell malignancies (14, 15). However, it may not be ideal for inducing CTL, due to potential competitive CTL-inducing epitopes in the lacZ gene. The poor performance of this design is confirmed by the relative failure of our gp70 fusion gene to influence antigen presentation via DNA vaccines. Vaccine 17:3030.


