Superior Tumor Protection Induced by a Cellular Vaccine Carrying a Tumor-
specific T Helper Epitope by Genetic Exchange of the Class II-associated
Invariant Chain Peptide

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ABSTRACT

Efficient loading of MHC class II molecules with a T helper epitope of choice can be achieved through genetic exchange of the MHC class II-associated invariant chain peptide (CLIP) sequence with a sequence encoding the helper peptide. We have now used this method to engineer a cellular vaccine that continuously expresses a tumor-specific helper epitope in a defined cosubstrium context. We provide evidence (a) that this cellular vaccine induces peptide-specific helper T cells in vivo that are functional in protecting mice from challenge with a highly aggressive tumor, (b) that this vaccine can directly prime tumor-specific helper T cells in vivo, and (c) that this cellular vaccine is superior compared with similar cells loaded with synthetic T helper peptide in inducing tumor protection. In conclusion, cellular vaccines for activation of antigen-specific helper T cells can be greatly improved by the introduction of invariant chain constructs containing a T helper epitope by class II-associated invariant chain peptide exchange.

INTRODUCTION

The activation of CD4+ helper T cells is essential to obtain specific systemic immunity. Helper T cells provide specific help to cytolytic T lymphocytes, antibody-producing B lymphocytes, and phagocytic cells. All of these forms of “help” are potentially involved in the eradication of tumor cells by the immune system (reviewed in Refs. 1–3). Thus, tumor immunotherapy protocols benefit from the concomitant induction of tumor-specific helper T cells, even in the case of class II-negative tumors (4, 5). In fact, in two mouse models the sole induction of tumor-specific helper T cells was sufficient to protect animals from subsequent tumor challenge (5, 6).

A central role for CD4+ T cells in tumor immunity emerged from studies of FMR3 MuLV type tumors (4). Protective immunity toward the MHC class II-negative FBL tumor cell line (a Friend MuLV-induced erythroleukemia cell line) could be transferred from immune mice to naive mice by purified CD4+ T cells. All of these forms of “help” are potentially involved in the eradication of tumor cells by the immune system (reviewed in Refs. 1–3). Thus, tumor immunotherapy protocols benefit from the concomitant induction of tumor-specific helper T cells, even in the case of class II-negative tumors (4, 5). In fact, in two mouse models the sole induction of tumor-specific helper T cells was sufficient to protect animals from subsequent tumor challenge (5, 6).

Central role for CD4+ T cell in tumor immunity emerged from studies of FMR3 MuLV type tumors (4). Protective immunity toward the MHC class II-negative FBL tumor cell line (a Friend MuLV-induced erythroleukemia cell line) could be transferred from immune mice to naive mice by purified CD4+ T cells. In the Rauscher MuLV model, a single s.c. vaccination with a synthetic Rauscher env/gp70-derived helper peptide in IFA protected, on average, 50% of the mice against subsequent challenge with the class II-negative tumor cell line RMA (5).

Optimal presentation of an epitope of choice for activation of helper T cells in vitro can be achieved by genetic exchange of CLIP with the helper peptide (7). This approach guarantees continuous and high-density expression of the T helper epitope on the surface of class II-positive APCs. We hypothesized that such a cell, provided it expresses the proper costimulatory signals, would be an efficient inducer of peptide-specific helper T cells in vivo. A well-controlled costimulatory context of the MHC class II-peptide complex is of importance, because antigen presentation in the absence of costimulation could cause T-cell tolerance. To this end, a cellular vaccine was created by transfecting a B-cell line expressing I-Ab, CD40, CD80, and CD86 with an li vector encoding the Rauscher MuLV T helper epitope in the position of the CLIP sequence. Mice were injected with this cellular vaccine, and the induction of peptide-specific helper T cells as well as the induction of tumor protection was evaluated. We show that the CLIP-engineered cellular vaccine directly primes tumor-specific helper T cells that protect animals from a lethal tumor challenge. Moreover, the level of protection is higher than that induced by the same cells loaded exogenously with the synthetic helper peptide.

MATERIALS AND METHODS

Mice and Cell Lines. C57BL/6 (H-2b) mice were obtained from IFA/CREDO (Rijswijk, the Netherlands) and bred under specific pathogen-free conditions at the Leiden University Medical Center animal facility. Female mice, 6–10 weeks of age, were used for all experiments. The nontumorigenic 771 B-cell lymphoma was derived from a C57BL/10 (H-2b) mouse that had been inoculated neonatally with MCF1233 MuLV (8, 9). MCF is immunologically distinct from FMR MuLV types and does not share any CTL or helper epitope with Rauscher MuLV (10). Transfectants of 771 were maintained in medium supplemented with 0.5 mg/ml hygromycin B (Boehringer Mannheim, Mannheim, Germany). RMA is a mutagenized derivative of RBL-5, a Rauscher MuLV-induced T-cell lymphoma cell line of C57BL/6 origin (11). The 3A12 helper T-cell clone was obtained from a C57BL/6 mouse vaccinated with the MuLV env/gp70-derived helper epitope (5). The LacZ inducible T-cell hybridoma BWZ36.1x3A12 was produced from 3A12 as described previously (12). All cell lines and bulk splenocytes were cultured in Iscove’s modified Dulbecco’s medium (Bio-Whittaker Europe, Verviers, Belgium) supplemented with 5% FCS (Greiner, Frickenhausen, Germany) and penicillin (100 units/ml), unless otherwise indicated.

Genetic Constructs and Transfections. A mouse Ii cassette vector was constructed in which the CLIP sequence can be replaced with sequences of choice (13). With reverse-transcribed cDNA from the 771 cell line as a template, the regions upstream and downstream of CLIP were amplified separately using primer pairs 5’-AACTGCTATCCATGGCCATGTGAAGATGCGGA-3’/5’-GGCATGAATTCCTTCGAAACAGGTTTGGCAGATTTC-3’ and 5’-CTTGGATTCCCGGGATGCTCATGGATAACATGTCTTCTG-3’/5’-GGATCCAGCTCGAGCCCTGCTTAC-3’/5’. The products of these PCRs were blunt-end phosphorylated and subsequently ligated into the pC20H vector. From these plasmids, the upstream region was isolated as a BamHI/EcoRI fragment, whereas the downstream region was isolated as an EcoRI/XhoI fragment. Both fragments were ligated into the multiple cloning site of pCMVΔN/Amp (Invitrogen, Leek, the Netherlands). The resulting gene construct encodes a modified Ii, which contains unique cloning sites SfiI and EcoRI in place of the CLIP-encoding sequence. Double-stranded oligonucleotides with sequences encoding either CLIP (QM-RM1P3LMR) or the antigenic core of the MuLV env/gp70-derived helper peptide (SLTRPCNTAWNR) were ligated into this cassette. The sequences of these oligonucleotides were as follows: (a) CLIP, 5’-CGCAGATGCCGGAG-3’.
TGGTACTCCTCCGTGATGCTC-3′/−GGCCGCATCAAGAACAGGAGTA-
GCCATCTCGTGAAG-3′; and (b) HELP, 5′-GGTCCCTCACCCTGCTGGT-
GCAAACGTCTGGAC-5′/−GGGCTGCTCAGGGCATTGTCGTGCA-
CCGGGTTGAGGA-3′. The resulting plasmids were termed pCLIP and pHELP, respectively.

NH₂-terminally truncated li deletion mutants lacking the first 59 amino acids of the recombinant li chains were generated by amplifying pHELP and pCLIP by PCR using primer pair 5′-AAACCTGATCCCTAGACCGTCTGCTGATAGACAAGTGTCGACC-3′/−GTCCTCTGAGTGCGGCTCTTGCTGCAACAAGTGTCGACC-3′. The PCR products were digested with BamH1/XhoI and ligated into pcDNA3/Amp. The resulting plasmids were termed pshHELp and pshCLIP. The plasmids were checked by sequencing in all cases.

Transfections were performed by electroporation. Briefly, 18 μg of pCLIP, pshCLIP, pcDNAI/Amp, or pshHELP plasmid and 2 μg of hygromycin resistance plasmid pTk hygro (14) were incubated with 5 × 10⁶ 771 cells in 400 μl of RPMI 1640/2% FCS for 10 min at room temperature. This suspension was transferred to a gene Pulser Cuvette (Bio-Rad, Hercules, CA). Electroporation was applied using a Bio-Rad gene pulser with the capacitance extender set at 960 microfarads and the voltage set at 300 V. Subsequently, the cells were cultured overnight in 10 ml of fresh medium. After 48 h, live cells were harvested from a Ficolli isopaque gradient and plated in a 96-well flat-bottomed plate at a concentration of 20,000 cells/well in the presence of 0.5 mg/ml hygromycin B. Single wells were harvested and expanded. Transfectants expressing the recombinant li were selected on the basis of recognition by the 3A12 T cell and/or reverse transcription-PCR and plated at 0.5 cell/well in the presence of 1000 γ-irradiated untransfected 771 cells/well and hygromycin B. The resulting transflectants were termed 771-CLIP, 771-HELP, 771-shCLIP, and 771-shHELP.

In Vitro T-Cell Assays. T-cell activation assays were performed by a 4-h or overnight incubation of APCs with 50,000 LacZ T-cell hybridoma cells in 96-well flat-bottomed plates before measurement of LacZ activity. After this incubation, total LacZ activity in individual wells was measured by lysing cells in 0.1 ml of Z buffer (100 mM 2-mercaptoethanol, 9 mM MgCl₂, and 0.125% NP40 in PBS) containing 0.15 mM chloroform red β-galactoside (Calbiochem). After a 4-h incubation at 37°C, the absorption at 595 nm was read using a 96-well plate reader.

Immunizations and Evaluation of Helper Activity. Mice received an i.p. injection of 10⁵ cells of the various live 771 transflectants in 0.2 ml of PBS. Ten days later, spleen cell suspensions were prepared and depleted of B cells using magnetic goat antimouse IgG-coated magnetic particles (PerSeptive Biosystems, Framingham, MA). The depleted splenocytes were cocultured with 100,000 γ-irradiated (3,000 rads) syngeneic spleen cells or wt helper cells in the presence of 10 μg/ml wt helper peptide. After 4 days of culture, [%H]Thymidine was added (0.5 μCi/well; 1 Ci = 37 GBq). [%H]Thymidine incorporation was measured 18 h later.

Necrotic cells were generated by three rounds of rapid freezing and thawing in liquid nitrogen (−180°C) and water (room temperature), respectively. Directly after the last cycle, 10⁴ necrotic 771-CLIP, 771-shHELP, or 771-HELP cells were injected s.c. in the flank of C57BL/6 mice. After 10 days, the animals were sacrificed, and their spleens were removed. B-cell-depleted spleen cells were restimulated once in vitro with 10 μg/ml wt helper peptide. After 10 days of in vitro culture, helper peptide-specific responses were evaluated by coculturing 12,500 cells from the bulk cultures with 100,000 γ-irradiated (3,000 rads) syngeneic splenocytes/well of a 96-well U-bottomed plate for 2 days in the presence or absence of 10 μg/ml helper peptide. IFN-γ production in the supernatants was measured by sandwich ELISA as described (15).

Tumor Protection Assays. Mice received an i.p. or a s.c. injection of the various live 771 transflectant APCs (10⁵ cells/mouse, unless otherwise indicated) in 0.2 ml of PBS. Peptide-loaded APCs were generated by adding 10 μg/ml helper peptide to the culture medium twice, 18 h and 2 h before the cells were harvested and washed in PBS for injection. For reference, mice received a single dose of synthetic peptide (50–100 μg/mouse) in a 50% (v/v) emulsion of PBS and IFA administered in a 0.2-ml depot s.c. After 14 days, the mice were challenged with 10⁵ RMA tumor cells administered in 0.2 ml of PBS, 0.1% (v/v) IFA. In some experiments, the mice were monitored regularly. Mice were killed if their weight increased >25% or were killed earlier if they showed obvious symptoms of tumor-related suffering, according to the guidelines of The Animal Experimentation Committee of the Leiden University.

Statistical analysis of the protection data was performed using the log-rank test. Significance was defined as P < 0.01.

Peptides. Peptides were generated by solid-phase synthesis on an AMBIMED 422 synthesizer (AMBIMED, Langenfeld, Germany) as described previously (16). Peptides were analyzed for purity by reverse-phase high-performance liquid chromatography and lyophilized. The env/gp70 T helper epitope EPLTSLTRCN2AWRLKL (17) was dissolved in PBS. The peptide encoded by the pHELP vector LPKSLKPVSSLPLRCN2AWNRPSMS (ll-helper peptide) and the gag-L-derived dominant CTL epitope CCLCVTVFL (18) were dissolved in DMSO and diluted in PBS.

RESULTS

Construction of a Transfectant APC. Efficient presentation of a T helper epitope in vitro can be achieved by transfection with an II-based vector in which CLIP is replaced with a helper epitope (7). We have now tested whether this method can be used to generate a cellular vaccine that efficiently primes tumor-specific T helper cells in vivo. For this purpose, we used the Rauscher MuLV-induced RMA tumor model. Rauscher MuLV contains an env/gp70-derived T helper epitope that is presented to helper T cells by I-Aα during immune responses against the FMR type of MuLV (17). Vaccination with this helper peptide induces long-term protective immunity toward RMA in approximately 50% of mice vaccinated (5). To generate a cellular vaccine, the 771 B-cell line was selected for its high surface expression of I-Aα and the costimulatory molecules CD40, CD80, and CD86 (data not shown). This cell line was transfected with an Ii vector encoding the core antigenic sequence of helper peptide instead of CLIP, yielding the stable transfectant 771-HELP. As a control, 771-CLIP was transfected with the wt Ii, which yielded 771-CLIP.

To test the I-Aα restricted presentation of the env/gp70 helper epitope embedded in the II, the T-cell stimulatory capacity of 771-HELP was analyzed. A T helper clone raised against the RMA helper peptide in vivo recognized 771-HELP, but not the control 771-CLIP transfectant (Fig. 1A). IIi-mediated peptide loading of the transfectant was optimal for the T cell because recognition could not be improved by the addition of synthetic helper peptide (Fig. 1A).

A potential advantage of transfection is the continuous generation of new antigenic complexes, whereas exogenously loaded synthetic peptide eventually dissociates from class II. To address this issue, the 771-CLIP control transfectant was cultured overnight with a high dose (10 μg/ml) of the helper peptide. The cells were washed and cultured in the absence of peptide. These peptide-loaded cells were used for stimulation of the T helper clone at 0, 1, 4, and 8 h after peptide incubation, and stimulation was compared with the endogenously loaded 771-HELP transfectant cultured under identical conditions. Within 1 h, the stimulatory capacity of the synthetic peptide-loaded cells had decreased by approximately 50% (Fig. 1B), and it was reduced to about 25% at t = 8 h, whereas the T cells continued to respond vigorously to the 771-HELP transfectant at all time points (Fig. 1B).

Induction of Peptide-specific Helper T Cells by Vaccination with 771-HELP. Next, we tested whether vaccination of mice with the 771-HELP transfectant activated helper T cell responses specific for the env/gp70 helper peptide. For this purpose, the 771-HELP vaccine was injected i.p. This administration route has been reported to be most efficient for direct priming of cytotoxic T cells (19). Two C57BL/6 mice were vaccinated i.p. with 10⁵ live 771-HELP cells, whereas two control C57BL/6 mice received the control transfectant 771-CLIP. B-cell-depleted splenocytes retrieved from these mice 10 days after vaccination were tested for peptide-specific responses (Fig. 2). The splenocytes from both mice vaccinated with the 771-HELP cells displayed peptide-specific proliferative responses, whereas the splenocytes from the 771-CLIP-vaccinated mice did not respond to
Vaccination with 771-HELP Induces Protection from Tumor Challenge. Next, we tested whether vaccination with 771-HELP would protect from challenge with a lethal dose of RMA tumor cells. Mice were vaccinated with the cellular vaccine i.p. and challenged with live RMA tumor cells 2 weeks later. Nonimmunized mice or mice vaccinated with 771-CLIP i.p. developed ascites within 3 weeks after tumor challenge (Fig. 3A). In contrast, 4 of 10 (40%) mice vaccinated i.p. with 771-HELP were protected from tumor outgrowth. In agreement with previous reports (5), s.c. vaccination with the helper peptide in IFA induced protection in 4 of 9 (44%) mice (data not shown). These results were confirmed in two additional, independent experiments (Fig. 3B and Fig. 5). To establish the optimal cellular vaccine dose for protection, mice were vaccinated i.p. with cell numbers ranging from $10^4$ to $2 \times 10^7$ 771-HELP cells (two doses are shown in Fig. 3B). The optimal level of protection was obtained by injecting $10^7$ 771-HELP cells i.p. (Fig. 3B). Thus, i.p. vaccination with 771-HELP reproducibly protected animals against tumor challenge. These results indicated that a single vaccination with 771-HELP induced peptide-specific helper T cells.

Vaccination with 771-HELP Can Directly Prime Helper T Cells. 771-HELP was designed to directly prime helper T cells because this would allow the endogenously synthesized antigen to be presented in a controlled costimulatory context. However, activation of naive T cells in many cases requires cross-presentation of antigen by specialized host APCs such as DCs. To distinguish between direct and indirect priming by 771-HELP, we generated an additional transfectant that endogenously expressed a similar amount of the helper antigen but was unable to directly present it to helper T cells. To this end, a truncated version of Ii-HELP was created that lacks the NH$_2$-terminal 59 amino acids. Because the NH$_2$-terminal sequence of the Ii contains endoplasmic reticulum and endosomal targeting sequences (20), the short Ii-HELP (shHELP) protein would remain cytosolic and would be unable to target the helper peptide into the peptide-binding groove of I-A$^\beta$ molecules on 771 cells. Indeed, the 771-shHELP transfectant failed to activate the highly sensitive peptide-specific LacZ T helper hybridoma *in vitro*, whereas already low numbers of 771-HELP cells activated the T-cell hybridoma (Fig. 4A).

To test whether 771-HELP and control transfectant 771-shHELP contained comparable amounts of antigen available for cross-presentation by host APCs *in vivo*, both 771-HELP and 771-shHELP were subjected to three rounds of rapid freezing and thawing immediately before vaccination. The resulting disrupted (“necrotic”) vaccine was no longer able to directly activate helper T cells (Fig. 4B), so that both vaccines could only use the indirect presentation pathway. We injected $10^7$ necrotic 771-CLIP, 771-HELP, or 771-shHELP cells s.c. (the pathway that favored cross-presentation of antigen; Fig. 5B). Ten days later, the spleens from these animals were taken for evaluation of peptide-specific T helper responses. The necrotic 771-shHELP and 771-HELP induced comparable peptide-specific IFN-$\gamma$ production (Fig. 4C), whereas 771-CLIP induced little or no IFN-$\gamma$. Hence, cross-priming control transfectant 771-shHELP was not recognized directly by helper T cells yet was able to generate as much antigen for cross-presentation *in vivo* as 771-HELP.

Next, the protective effects of vaccination with live 771-shHELP versus 771-HELP were compared. All mice vaccinated with 771-HELP induced peptide-specific helper T cells, whereas already low numbers of 771-HELP cells activated the T-cell hybridoma *in vitro*, whereas already low numbers of 771-HELP cells activated the T-cell hybridoma (Fig. 4A).

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shHELP i.p. developed a tumor, but 771-HELP injected i.p. significantly delayed tumor outgrowth and induced long-term protection in 4 of 12 mice (Fig. 5A). In the same experiment, s.c. injection of 771-shHELP did delay tumor growth and protected 2 of 12 of mice, as compared with 10 of 12 for 771-HELP (Fig. 5B). This experiment showed that 771-shHELP did contain the helper peptide in amounts sufficient to raise antigen-specific helper T cells. In conclusion, these results indicate that the protective effect of i.p. vaccination with 771-HELP was mediated via direct presentation to the immune system, whereas s.c. vaccination favored the indirect presentation pathway.

Endogenous Ii-mediated Loading Is Superior to Exogenous Loading with Synthetic Peptide. In vitro, the stimulatory capacity of 771-HELP transfectant remained constant over time, whereas the antigenicity of 771-CLIP loaded with synthetic peptide was lost rapidly (Fig. 1B). Therefore, the induction of protection by endogenously loaded 771-HELP cells was compared with the protective capacity of exogenously loaded 771-CLIP control cells. Both the i.p. and s.c. administration pathways were used (Fig. 5A and B). Vaccination showed that 771-HELP cells maintained the helper peptide in amounts sufficient to raise antigen-specific helper T cells. In conclusion, these results indicate that the protective effect of i.p. vaccination with 771-HELP was mediated via direct presentation to the immune system, whereas s.c. vaccination favored the indirect presentation pathway.

Fig. 3. Vaccination with 771-HELP protects against subsequent tumor challenge. Mice were vaccinated i.p. with the indicated number of live 771-HELP cells or control live 771-CLIP cells and challenged with 1000 live RMA tumor cells i.p. 2 weeks later. The development of ascites (Y axis) was followed as a measure of outgrowth of the RMA cells. The results of two separate experiments are shown (A and B). Significance of differences was analyzed by the log-rank test. A: 771-CLIP versus 771-HELP, P = 0.004; nonimmunized versus 771-HELP, P = 0.0006; nonimmunized versus 771-CLIP, P = 0.27 (not significant). For B, 10^6 and 2 × 10^7 771-CLIP cells were pooled and compared with 10^6 771-HELP (P = 0.0008) and 10^7 771-HELP (P = 0.0007).

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In conclusion, Ii-mediated peptide loading was superior to exogenous loading of synthetic peptide in inducing protective immunity by both the i.p. and s.c. routes of administration.

DISCUSSION

Continuous high density and functional expression of a T helper epitope can be achieved through genetic exchange of the CLIP. In this study, we show for the first time that this method can be applied to engineer a cellular vaccine that induces functional antigen-specific T helper cells by direct priming. Moreover, vaccine cells loaded in this manner were superior to the same cells loaded with synthetic peptide in inducing protective immunity against the highly aggressive virus-induced RMA tumor.

In our previous study (5), we showed that protection induced by vaccination with the synthetic env/gp70 helper peptide could be abrogated by in vivo anti-CD4 treatment at the time of vaccination and by anti-CD4 or anti-CD8 treatment just after RMA challenge. Because RMA does not express class I, env/gp70-specific helper T cells do not lyse RMA in vitro (5). RMA also does not express class II after i.p. injection and in vivo expansion in mice (5). Therefore, these studies suggest that on challenge with live RMA tumor cells, tumor antigens are processed and presented by specialized APCs. The preexistence of tumor-specific helper T cells induced by vaccination with helper peptide would then allow activation and persistence of tumor-specific CTLs that are directly responsible for eradicating the RMA tumor (5). The protective effect of our cellular vaccine is likely to be based on a similar mechanism because env/gp70 helper peptide-specific proliferative responses could be recovered from mice on vaccination with our cellular vaccine 771-HELP, but not from mice that had been vaccinated with control cells (Fig. 2).

The data underline the importance of the costimulatory context of antigen presentation in deciding between tolerance and activation. The i.p. vaccination with synthetic helper peptide elicits antigen-specific tolerance (21). In addition, synthetic peptide vaccination protocols
have been revealed to be rather unpredictable. Identical vaccination schemes have led to either effective immunization or tolerization, depending on the peptide dose, the physico-chemical characteristics of the peptide, and the delivery route (21–23). Our approach uses the natural MHC class II assembly route to deliver a defined helper peptide to the class II molecules on the surface of cells expressing the appropriate costimulatory molecules. On i.p. administration of the cellular vaccine, the helper peptide was only presented in this controlled context because indirect presentation of the antigen did not contribute to protection (Fig. 5A). Moreover, when the cellular vaccine was injected s.c. (Fig. 5D), which allowed both direct and indirect presentation to the immune system, the level of protection (83%) was higher than ever observed with synthetic helper peptide in IFA injected s.c.5 Extensive studies by our group have established that a maximum of 50% protection can be achieved by helper peptide vaccination. The failure to protect all mice could be due to a failure to effectively prime a sufficiently large number of tumor-specific helper T cells in some animals.

An intriguing finding was that helper peptide-specific IFN-γ responses were retrieved from mice vaccinated with necrotic cells (Fig. 4C). Thus far, vaccination with necrotic cells does not appear to induce a CTL response (24). When necrotic cells were used as adjuvant in combination with ovalbumin, ovalbumin-specific delayed type hypersensitivity responses were detected (25). This result indicated that vaccination with necrotic cells induced helper responses directed against antigens present in the necrotic lysate. Our findings show that vaccination with necrotic cells can induce T helper responses against an intracellular antigen (Fig. 4C).

To the best of our knowledge, there is only one other set of studies in which it has been shown that endogenous targeting of antigens to the MHC class II presentation pathway can induce protective immunity toward tumors. Wu and Pardoll describe a vaccinia construct containing a full-length tumor antigen (human papillomavirus type 16 E7) joined to a lysosome-associated membrane protein (LAMP-1) targeting sequence (26–28). This viral vaccinia construct containing a full-length tumor antigen (human papillomavirus type 16 E6 and E7 and c-Ha-ras oncogenes). In vivo studies in which it has been shown that endogenous targeting of responses against an intracellular antigen (Fig. 4) show that vaccination with necrotic cells can induce T helper re-

REFERENCES
Superior Tumor Protection Induced by a Cellular Vaccine Carrying a Tumor-specific T Helper Epitope by Genetic Exchange of the Class II-associated Invariant Chain Peptide

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