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Th2-Type CD4+ Cells Neither Enhance nor Suppress Antitumor CTL Activity in a Mouse Tumor Model

Germain J. P. Fernando, Trina J. Stewart, Robert W. Tindle, and Ian H. Frazer

Many cervical cancers express the E7 protein of human papillomavirus 16 as a tumor-specific Ag (TSA). To establish the role of E7-specific T cell help in CD8+ CTL-mediated tumor regression, C57BL/6J mice were immunized with E7 protein or with a peptide (GF001) comprising a minimal CTL epitope of E7, together with different adjuvants. Immunized mice were challenged with an E7-expressing tumor cell line, EL4.E7. Growth of EL4.E7 was reduced following immunization with E7 and Quil-A (an adjuvant that induced a Th1-type response to E7) or with GF001 and Quil-A. Depletion of CD8+ cells, but not CD4+ cells, from an immunized animal abrogated protection, confirming that E7-specific CTL are necessary and sufficient for TSA-specific protection in this model. Immunization with E7 and Algammulin (an alum-based adjuvant) induced a Th2-like response and provided no tumor protection. To investigate whether a Th2 T helper response to E7 could prevent the development of an E7-specific CTL-mediated protection, mice were simultaneously immunized with E7/Algammulin and GF001/Quil-A or, alternatively, were immunized with GF001/Quil-A 8 wk after immunization with E7/Algammulin. Tumor protection was observed in each case. We conclude that an established Th2 response to a TSA does not prevent the development of TSA-specific tumor protective CTL. The Journal of Immunology, 1998, 161: 2421–2427.

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4 Abbreviations used in this paper: HPV, human papillomavirus; TSA, tumor-specific Ag; GST, glutathione S-transferase.

The tumor protective effect of a Th1-type response has also been reported in several studies involving spontaneously regressing tumors. A recent study has shown that in spontaneously regressing primary melanomas, the mRNA levels for Th1-type cytokines (IL-2, TNF-α, and INF-γ) were increased compared with non-regressing melanomas (8). Levels of Th2-type cytokines IL-10 and IL-13 did not differ. In another study on anogenital warts (9), it was found that the mRNA expression patterns in 7 of 8 nonrecurrent warts were compatible with a predominant Th1 or mixed Th1/Th2 cytokine profile, whereas in recalcitrant warts, Th1-like cytokine mRNA expression was not detected. Furthermore, in the recurrent warts of a renal transplant patient, increased IL-4 and IL-5 mRNA expression was found repeatedly, suggesting a predominant Th2-type response. These data indicate, in general, that tumor regression is favored by Th1-type cytokines and that Th2-type cytokines may have the opposite effect. This type of protective Th1 response has also been observed in other disease states (10–15).

Immunotherapy targeted at the HPV16 E7 protein in a vaccine with an adjuvant known to induce a strong Th1-type response may therefore enhance tumor-specific immunity in cervical cancer patients and may lead to tumor regression. In the present study, we have used a mouse tumor model, EL4 thymoma transfected with the HPV16 E7 gene, to determine whether a preexisting Th2-type immune response to HPV16 E7 protein would preclude development of an E7-specific CTL response following appropriate immunization. This type of mouse tumor model has been used extensively by others (16–19) to study different aspects of experimental cervical cancer immunology.

Materials and Methods

Mice

C57BL/6J female mice were obtained from the Animal Resources Centre, Perth, Australia. The mice were maintained under specific pathogen-free conditions and were used at 6 to 10 wk of age. Genetic authenticity of these mice was checked at regular intervals.
Peptides, proteins, and adjuvants

Peptide GF001, comprising the H-2D^d-restricted minimal CTL epitope (17, 20) of HPV16 E7 protein, sequence RAHYNIVTF (single-letter amino acid code), was synthesized as described previously (21). Purity of the peptide was >95% by reverse phase HPLC (22), and the peptide was used without further purification. Synthetic peptide corresponding to amino acids 44–62 of E7 protein (8Q: sequence QAEPDRAHYNTFCCKCD) and its CTL epitope mutant peptide analogue (MT106: sequence QAEP DRAHYNTACCKCD) were synthesized as end-blocked peptides by ChemTech Sciences (Clayton, Australia). OVA (A-A5503) was purchased from Sigma Chemical (St. Louis, MO). HPV16 E7 protein was produced as a glutathione S-transferase (GST) fusion protein in Escherichia coli and obtained at a purity of >85% by using ion exchange chromatography (Fernando et al., manuscript in preparation). Purified protein was substantially freed of endotoxin activity by a Triton X-114 (Fluka, Neu-Ulm, Switzerland) extraction step (23), cleaned of Triton-X114 by acetone extraction, and extensively dialyzed against 5 mM HEPES, 150 mM NaCl (pH 7.2) buffer. Endotoxin levels observed were <100 endotoxin units/mg protein using a chromogenic Limulus amebocyte lysate assay (QLC-1000; Bio-Whittaker, Walkersville, MD).

Recombinant E. coli-producing HPV16 E6 and E7 MS2 fusion proteins, a gift of Dr. K. Seedorf, were isolated and purified as described previously (24). Quil-A (Spikoside) (25) was from Iscotec (Sweden Lulea); CFA and IFA were purchased from Sigma Chemical; and Algammulin (γ-interleukin-adsorbed alum) (26) was kindly provided by Dr. Peter Cooper (Australian National University, Canberra, Australia).

Tumor protection assays

Tumor cell lines and their growth conditions were as previously described (17, 27). Mice (8 to 10 per group) were immunized s.c. at the base of the tail with 50 μg of E7GST protein, or 50 μg OVA as control, and 10 μg of Quil-A or 50 μg of Algammulin as adjuvant. Mice were challenged with 3 × 10^5 cells of EL4.E7 tumor (27) or 2 × 10^5 cells of C3 cells (17) 14 days after the last immunization. Fourteen days after tumor challenge, the mice were killed and tumor tissue weighed. In some experiments, mice were checked for palpable tumor every other day and the data recorded. The experiments were repeated at least twice.

Assay of cytotoxic T cell activity

CTL activity was measured using a standard 5-h 3^51Cr release assay, with EL4, EL4/E7, and EG7.OVA cells as targets. Effector cells were obtained by culturing draining lymph node cells with 20 U/ml IL-2 for 4 days in RPMI complete medium. Control effector cells, prepared from mice immunized with OVA and the appropriate adjuvant, were similarly restimulated in vitro. EG7.OVA, EL4 cells transfected with an OVA expression plasmid, were a gift from Dr. Francis Carbone (Monash Medical School, Prahran, Australia).

Depletion of CD4^+ CD8^+ cells in mice

mAb to CD4 (GK1.5, ATCC cell line 207-TIB, batch F-12087; American Type Culture Collection (ATCC), Manassas, VA) (28) and CD8 (2.43, ATCC cell line 210-TIB, batch F-11370) (29) were prepared as ascites in BALB/c nu/nu ("nude") mice. mAb were partially purified by 45% ammonium sulfate precipitation and then extensively dialyzed against 20 mM sodium phosphate, 100 mM NaCl (pH 7.2) buffer (PBS).

Mice were immunized with 50 μg of E7GST protein and 10 μg of Quil-A at day 0 and 21, and at day 28, 32, and 38 were given 1 mg of the appropriate Ab i.p. in 2 ml of PBS. Mice were challenged with EL4.E7 tumor at day 35 and killed on day 49.

The level of CD4^+ and CD8^+ cells after in vivo depletion by GK1.5 and 2.43 mAb, respectively, was measured by FACS analysis using different mAbs. FITC-labeled anti-mouse CD4 clone H-129.19 (Sigma Chemical, cat. no. F-7400) was used to determine the levels of CD4^+ cells. Anti-OVA FITC (Serotec, Oxford, U.K.) (2422) was used as isotype control Ab. Anti-mouse CD8^+ clone 53-6.7 labeled with R-phycocerythrin (R-PE, Sigma cat. no. P-3607) was used to determine the levels of CD8^+ cells. Anti-OVA phycoerythrin (Serotec, cat. no. MCA1212PE) was used as isotype control Ab in this experiment. To further study the effect of the depletion of CD4^+ cells on tumor regression at different time points (i.e., before immunization and postimmunization), the mice were immunized as above and the anti-CD4 Ab was given at either 7 days before the first immunization and then at day 14 and 24, or 7 days before tumor challenge and 4 days after tumor challenge. In these experiments, C3 cells (17) were used in the tumor challenges.

Measurement of Ab response

Ab to E7 protein was measured by ELISA as previously described (30). Briefly, the ELISA plates were coated with HPV16 E7 MS2 fusion protein and were used to determine the anti-E7 Abs elicited. HPV16 E6 MS2 fusion protein-coated ELISA plates were used as controls. The color development was performed using ABTS (2,2′-azino-bis[3-ethylbenzthiazolone-6-sulfonic acid]) (Sigma cat. no. A-1888) as substrate, and the OD readings at 415 nm were measured using a Bio-Rad (Hercules, CA) model 3550 plate reader. Each sample was analyzed in triplicate, and the OD readings were expressed as mean ± SEM.

Results

HPV16 E7 protein is a tumor-specific Ag when expressed in the EL4 thymoma cell line

We have previously described the construction of an HPV16 E7-transduced EL4 thymoma cell line (EL4.E7), which grows in syngeneic mice as discrete s.c. nodules, and shown that immunization with E7 in the form of ISCARS (immunostimulatory carriers) reduces tumor growth (27). To confirm that tumor growth retardation following immunization with E7 is E7 specific, groups of mice were immunized with E7 plus Quil-A or, as control, with OVA plus Quil-A, then challenged with EL4 or EL4.E7 cells. Tumor cells were first given at a dose of 3 × 10^6 cells/mouse, the smallest quantity that produced palpable tumors in 10 of 10 immunized mice within 20 days after challenge. Mice immunized with E7 and challenged with EL4.E7 were substantially tumor free 80 days after challenge (1 of 10), whereas 10 of 10 mice immunized with OVA/Quil-A and 10 of 10 mice immunized with E7/Quil-A and challenged with the parent EL4 cell line developed palpable tumors, as expected, 14 to 20 days after tumor challenge (Fig. 1). To minimize animal distress and experiment duration, mice in subsequent experiments were challenged with a larger dose of tumor (3 × 10^6) such that tumor growth was observed over the first 2 wk following challenge in every animal, but was significantly retarded if the animals were optimally immunized. Animals were sacrificed 14 days after tumor challenge, and the tumors were weighed; retardation of growth compared with control mice was then used as a measure of tumor-specific immunity in each experiment.

Tumor protection is mediated by CD8^+ cells

To determine the role of CD4^+ and of CD8^+ T cells as mediators of tumor protection in this model, mice were immunized with E7/ Quil-A to induce a tumor protective immune response, and tumor growth was compared for untreated mice and mice treated with mAb to deplete CD4^+ and/or CD8^+ cells before tumor challenge. Mice treated with anti-CD8 mAb (2.43) (29) were demonstrated by

FIGURE 1. Groups of 10 C57BL/6J mice were immunized twice, at day 0 and 21, with 50 μg of E7GST protein (or with OVA as a control) and 10 μg Quil-A, then challenged with 3 × 10^5 tumor cells (EL4. E7) 14 days after the last immunization. Mice were checked at regular intervals for palpable tumor. Mice with palpable tumors were killed and the data recorded. The number of mice free of tumor was plotted against the dates checked after the tumor challenge.

Measurement of Ab response

Ab to E7 protein was measured by ELISA as previously described (30). Briefly, the ELISA plates were coated with HPV16 E7 MS2 fusion protein and were used to determine the anti-E7 Abs elicited. HPV16 E6 MS2 fusion protein-coated ELISA plates were used as controls. The color development was performed using ABTS (2,2′-azino-bis[3-ethylbenzthiazolone-6-sulfonic acid]) (Sigma cat. no. A-1888) as substrate, and the OD readings at 415 nm were measured using a Bio-Rad (Hercules, CA) model 3550 plate reader. Each sample was analyzed in triplicate, and the OD readings were expressed as mean ± SEM.

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FACS analysis to have <1% CD8+ T cells in the spleen, and mice depleted with anti-CD4 mAb (GK1.5) (28) had <1.1% CD4+ T cells. Mice treated with control Abs showed no perturbation of the CD4+ or CD8+ T cell numbers. In two independent experiments, E7-immunized mice depleted of CD8+ cells, or of both CD8+ and CD4+ cells, were as susceptible to the EL4.E7 tumor as immunized mice. In contrast, mice depleted of CD8+ cells or of CD4+ cells and control mice were tested for Ab to E7, and similar levels were seen in the protected and unprotected mice. Serum reactivity to E7 by ELISA (pooled sera for each group, shown as mean OD415 nm ± SEM) was similar at the time of sacrifice in immunized mice with no depletion of CD4+ cells (0.806 ± 0.006) to that in CD4+ cell-depleted mice (0.819 ± 0.009) and CD8+ cell-depleted mice (0.857 ± 0.008) or CD4+ plus CD8+ cell-depleted mice (0.860 ± 0.008)). These results suggest that tumor protection in this system requires E7-specific CD8+ cells and is not mediated solely by CD4+ cells or by Ab to E7.

To further investigate the role of CD4+ cells, experiments were performed with cell depletion at different time intervals, i.e., before immunizations and before tumor challenge. In these experiments also, the results indicate (Fig. 2B) that the depletion of CD4+ cells either before and during immunizations or immediately before tumor challenge has no effect on the tumor protection induced by the vaccine. Mice depleted of CD4+ cells before immunization were functionally deprived of E7-specific T help, as four of five mice depleted failed to develop Ab to E7 (group 4, G STR-specific ELISA OD415 nm at the day of tumor challenge: 0.029, 0.002, 0.003, 0.013, and 0.597), in comparison with mice immunized with E7 and sham depleted with the control Ab (group 5, E7-specific ELISA OD415 nm at the day of tumor challenge: 1.270, 1.501, 1.282, 0.986, and 1.886).

FIGURE 2. A, C57BL/6J mice (10 per group) were immunized twice, at day 0 and 21, with 50 μg of E7GST protein and 10 μg Quil-A (groups 1–5) or were left unimmunized (group 6). At day 28, 32, and 38, mice were injected i.p. with 1 mg of the appropriate mAb: group 2, irrelevant Ab (rat IgG); group 3, anti-CD4; group 4, anti-CD8; and group 5, anti-CD4/CD8. Mice were challenged with 3 × 10^6 tumor cells (EL4. E7) at day 35. The mice were killed at day 49 and the tumor weight recorded. B, C57BL/6J mice (10 per group) were immunized twice, at day 0 and 21, with 50 μg of E7GST protein and 10 μg of Quil-A (groups 1–5) or immunized with OVA and Quil-A as control (group 6). At day 56 and 67, mice from group 2 and 3 were injected i.p. with 1 mg of the appropriate mAb: group 2, anti-CD4; group 3, irrelevant Ab (rat IgG). Seven days before the first immunization and at day 14 and 24, mice from group 4 and 5 were injected i.p. with 1 mg of the appropriate mAb: group 4, anti-CD4; group 5, irrelevant Ab (rat IgG). Mice were challenged with 2 × 10^6 tumor cells (C3) at day 63. The mice were killed at day 77 and the tumor weight recorded. C, Groups of 10 C57BL/6J mice were immunized twice, at day 0 and 21, with different immunogens (group 1, Nil; group 2, Quil-A; group 3, E7GST/Quil-A; group 4, GF001/Quil-A) and challenged with 3 × 10^6 tumor cells (EL4. E7) 14 days after the last immunization. Two weeks after the tumor challenge, mice were killed and the tumor weights recorded. D, Groups of 10 C57BL/6J mice were immunized twice, at day 0 and 21, with different immunogens (group 1, Quil-A; group 2, GF001/Quil-A; group 3, 8Q/Quil-A; group 4, MT106/Quil-A; group 5, E7GST/Quil-A) and challenged with 3 × 10^6 tumor cells (EL4. E7) 14 days after the last immunization. Two weeks after the tumor challenge, mice were killed and the tumor weights recorded. GF001, minimal CTL epitope peptide; 8Q, 19-mer peptide containing the major T helper and the CTL epitope; and MT106, the 8Q analogue in which the CTL epitope has been disabled with a mutation in the anchor residue.
The IgG2a/IgG1 ratio was not predictive of the degree of tumor mass, between individual mice (Fig. 3) (p = 0.343, r² = 0.030; using a linear regression, least squares method), suggesting that the cytokine environment provided by TSA-specific Th cells, although a determinant of CTL induction, is not a determinant of CTL-mediated tumor protection once TSA-specific T cells are induced.

Tumor protection mediated by CTLs-specific for the CTL epitope RAHYNIVTF

To confirm that tumor protection in this model is mediated by MHC class I-restricted CTLs, mice were immunized with a peptide comprising the major (Dα-restricted) CTL epitope of HPV16 E7 (GF001 = RAHYNIVTF, single-letter amino acid code), which has been previously shown to lack T helper/T proliferative epitopes (17), and were challenged with tumor as before. Mice immunized with the minimal CTL epitope were protected against tumor growth to an extent similar to mice immunized with E7 (Fig. 2C), which suggests that CTLs alone without specific T cell help or Ab could function as effective tumor killers.

An E7 peptide in which the anchor residue of the CTL epitope is mutated does not promote tumor regression

To confirm that tumor regression is mediated by RAHYNIVTF-specific CTL, groups of mice were immunized with a synthetic peptide with the minimal CTL epitope alone (GF001) or, alternatively, with a peptide including the minimal CTL epitope and the major T helper epitope of E7 protein (8Q), or with an 8Q peptide analogue (MT106) in which the hydrophobic anchor residue, phenylalanine, of the CTL epitope was changed to lysine, a charged residue, to deactivate the CTL epitope. E7 protein was used as a positive control. Tumor growth was inhibited in animals immunized with GF001 or 8Q, but not in animals immunized with MT106 (Fig. 2D), showing that that the tumor regression depends on an intact CTL epitope RAHYNIVTF.

Comparison between tumor protection and the nature of induced immunity

We have previously compared a range of adjuvants for their efficacy at inducing an E7-specific cytotoxic T cell response measured in vitro using 51Cr-labeled EL4.E7 cells as targets and have demonstrated a correlation of the E7-specific CTL precursor response with the ratio of E7-specific IgG2a to E7-specific IgG1 Ab in serum (31). Immunization with E7 together with Quil-A effectively induced E7-specific CTL precursors that could be measured in vivo in vitro assay, whereas immunization with E7 and CFA or with E7 and Algamulin, an alum-based adjuvant with a high ratio of alum to y-inulin, induced only minimal CTL reactivity. In keeping with this observation, mice immunized with E7 + CFA or E7 + Algamulin, in contrast to mice immunized with E7 + Quil-A, showed unreduced susceptibility to the EL4.E7 tumor (Table I).

To determine whether the anti-E7 IgG2a/IgG1 ratio was predictive of tumor mass, mice were immunized with E7 together with different adjuvants that produced a range of IgG2a/IgG1 ratios. The IgG2a/IgG1 ratio was not predictive of the degree of tumor mass, between individual mice (Fig. 3) (p = 0.343, r² = 0.030; using a linear regression, least squares method), suggesting that the cytokine environment provided by TSA-specific Th cells, although a determinant of CTL induction, is not a determinant of CTL-mediated tumor protection once TSA-specific T cells are induced.

Tumor protection effect of GF001/Quil-A immunization is not abrogated by simultaneous immunization with E7/Algamulin

To confirm that a Th2-type T helper response to E7 had no effect on induction of E7-specific tumor regression, mice were immunized with GF001/Quil-A in the tail base and simultaneously immunized with E7/Algamulin s.c. on the rump. The Th2-type response induced by E7/Algamulin (31) had no effect on GF001/Quil-A-induced tumor protection (Fig. 4). Similarly, simultaneous immunizations of GF001/Quil-A and E7/Quil-A to induce a Th1-type helper response (31) also showed no effect on the tumor protection. Serum IgG2a reactivity to E7 by ELISA (expressed as OD415 nm ± SEM) was significantly higher in E7/Quil-A-immunized mice (IgG2a = 0.233 ± 0.005; IgG1 = 0.615 ± 0.040) than in E7/Algamulin-immunized mice (IgG2a = 0.022 ± 0.010; IgG1 = 0.437 ± 0.037).

A preexisting Th2-type response to E7 protein does not suppress generation of E7-specific CTL or tumor protection

Development of Ag-specific CTL is said to require a cytokine environment of Th1 type, whereas Th2-type cytokines are supposed to produce a specific response dominated by Ab and CTL suppression. To establish whether a preexisting Th2-type response to E7 protein would prevent induction of CTL-mediated tumor protection, mice were immunized on day 0 and 21 with E7 protein

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**Table I. Influence of adjuvant on tumor protection and class Ab response**

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<th>Immunogen</th>
<th>Total IgG</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgG2a/IgG1 ratio</th>
<th>CTL activity E:T = 40:1</th>
<th>Tumor weight (g)</th>
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**Figure 3.** Individual mouse sera were diluted 1:100 and analyzed for anti-E7 IgG2a and IgG1 reactivity by ELISA using plates coated with HPV16 E7 MS2 fusion protein. The anti-E7 IgG2a/IgG1 ratio was plotted against the tumor weight observed. Plates coated with HPV16 E6 MS2 protein were used as control.
Fig. 4. Groups of 10 C57BL/6J mice were immunized twice, at day 0 and 21, with different immunogens (group 1, Quil-A; group 2, GF001/Quil-A; group 3, GF001/Quil-A and E7/Quil-A; group 4, GF001/Quil-A and E7/Algammulin) and challenged with 3 × 10⁶ tumor cells (EL4.E7) 14 days after the last immunization. Two weeks after the tumor challenge, mice were killed and the tumor weights recorded. Peptides were immunized s.c. at the tail base, and E7 protein was simultaneously injected s.c. on the rump.

Fig. 5. Groups of 10 C57BL/6J mice were immunized on day 0 and 21 with different immunogens (group 1, None; group 2, None; group 3, E7/Quil-A; group 4, E7/Algammulin), and groups 2 to 4 were also immunized at day 35 and 56 with the minimal CTL epitope peptide GF001 and Quil-A. On day 70, the mice were challenged with 3 × 10⁶ EL4.E7 tumor cells, and on day 84 they were killed and the tumor weight was recorded.

Together with Algammulin or, alternatively, with E7 + Quil-A as a Th1 control. Mice were immunized at day 35 and 56 with the minimal CTL epitope peptide GF001 and Quil-A. The Th2-type response induced by the E7/Algammulin did not abrogate the ability of GF001/Quil-A to induce tumor protection (Fig. 5.). At the termination of the experiment, high levels of E7-specific IgG2a in the E7/Quil A group (IgG2a = 0.314 ± 0.031; IgG1 = 0.647 ± 0.067) and low levels in the Algammulin group (IgG2a = 0.029 ± 0.006; IgG1 = 0.647 ± 0.059) confirmed that a Th1 (E7/Quil A)- or Th2-type response (E7/Algammulin) to E7 had been established in these animals (31). In a similar experiment, preimmunization with E7/CFA had no positive or negative effect on the tumor regression induced by subsequent immunization with GF001/Quil-A (data not shown). We conclude that neither preexisting Th1 or Th2 cells specific for E7 influence the induction of E7-specific CTL-mediated tumor protection in this model.

Discussion

We have used a mouse tumor transfected with the E7 oncoprotein of cervical cancer-associated HPV as the TSA to study tumor regression mechanisms in response to an E7 vaccine. Tumor regression was observed when mice were immunized with E7 protein and Quil-A adjuvant. This protection is abrogated by depletion of CD8⁺ cells; however, no difference in protection was seen when CD4⁺ cells were depleted. Immunization with a minimal CTL epitope of the TSA, lacking functional T helper activity (17), inhibited tumor growth as effectively as immunization with the whole TSA. Maximal tumor protection occurs, therefore, in this model in the absence of Ag-specific T help or of Ab, and TSA-specific CTL can be held to be both necessary and sufficient to produce maximal protection. We have also shown that a preexisting Th2-type helper response to the tumor-specific Ag E7 does not prevent the development of an E7-specific antitumor CTL activity.

This study confirms the observation made in a different model that immunization with a minimal CTL epitope peptide of HPV16 E7 can induce an immune response that protects mice against an E7-expressing tumor (17). Adoptive transfer experiments with CTL clones have similarly shown that CTL alone could mediate tumor regression in a model in which adenovirus E1a protein was used as a virus-specified TSA (32). We recently showed, using a synthetic CTL polyepitope DNA vaccine, that the CTL response generated does not depend on CD4 T cell-mediated help (33). We have shown that when the minimal E7 CTL epitope is covalently attached to a foreign T helper protein, tumor regression is also observed (34). The lack of need for cognate T help for CTL effector function in these models may reflect the ability of TSA-specific CTL to secrete their own IL-2 (35, 36), which is presumably a reflection of the relative “strength” of viral proteins as TSAs.

In contrast to an apparent lack of need for TSA-specific T help in the effector phase of the immune response to tumors, T help in the induction phase of CTL-mediated tumor regression is often required. Significant inhibition of tumor growth was shown when mice were immunized with OVA 2 to 3 days after challenge with an OVA-transfected cell line (37). While depletion of CD8⁺ cells before immunization completely abrogated tumor inhibition, depletion of CD4⁺ cells also led to loss of tumor protection. However, in our tumor model system, no differences were seen in tumor protection even when CD4⁺ cells were depleted before immunization. In a model using an epithelial cell line transformed with HPV16 E6 and E7 and c-Ha-ras oncopgene (18), tumor regression was observed only when E7 was attached to a signal sequence that facilitates entry into class II pathway, and both CD4⁺ and CD8⁺ T cells were required for induction of protective immunity. The lack of requirement of T help for induction of tumor protective CTL, as demonstrated by the ability of a minimal CTL epitope lacking Th function to induce maximal protection in our model, may therefore reflect induction of CTL, which secretes cytokines more readily, particularly as Quil-A adjuvant may activate local cytokine secretion (38).

Several studies have examined the role of cytokine secretion by Th cells in influencing induction of CTL activity. Purified mouse anti-influenza CD8⁺ cells were unable to generate CTL activity in vitro in the absence of CD4⁺ Th cells (39). Both Th1- and Th2-type cells or cell-free supernatants derived from these cells provided help by secreting IL-2 and IL-4, respectively, to generate cytotoxic activity. Th1-type T helper clones promoted significantly stronger CTL responses than Th2 clones. Furthermore, cell-free supernatant from activated Th2-type clones had an antagonist effect on the generation of a CTL response by Th1 clones; this could be reversed by the addition of excess rIL-2. An inhibitory effect of Th2 T helper on virus clearance has been similarly demonstrated in vivo (40, 41). Adoptive transfer of a Th2-like cell line specific for alloantigen prolonged MHC class II Ag disparate skin allograft survival in mice, suggesting in another model that induction of CTL effector function may be down-regulated by the local secretion of cytokines by Th2-type cells in response to antigenic challenge.
(42). It was therefore of considerable interest to determine the effect of TSA-specific T help, and in particular of Th1- and Th2-type T help, on the induction and function of CTL in a tumor model in which there was no absolute need for T help for CTL induction or CTL function. From the present study, we conclude that preexisting TSA-specific Th status, whether Th1 or Th2, does not alter the maximal tumor regression induced by immunization with a minimal CTL epitope. As the adjuvant used for delivery of tumor Ag was nevertheless a major determinant of the protection that could be invoked, the ability of the adjuvant to directly elicit Ag-specific effector CTLs rather than its ability to promote particular cytokine secretion is the critical determinant of the effectiveness of the protection induced by immunization. This effect of Quil-A may be mediated by facilitation of Ag entry into a class I presentation pathway in the regional lymph node (43).

These findings complement studies in which the nature of the immune response to an Ag is shown to be determined by prior exposure of the animal to Th1- or Th2-promoting adjuvants given with an irrelevant Ag (44) and confirm that this effect may occur only if animals are exposed early enough to the immunogen producing the Th1 or Th2 bias. It may be that the immune system is programmed through genetic and/or early environmental effects to be able to produce or, alternatively, not produce a CTL response to novel Ags. Whether an animal set up to produce CTL then fulfills this potential on contact with Ag will be determined by the mode of Ag delivery and particularly its effective delivery to the class I compartment for presentation.

The data in this study indicate that even though cervical cancer patients may have a preexisting Th2-type of T helper response to the E6 and E7 oncoproteins (7), it may be possible to induce a tumor protection response in these patients by vaccination with suitable adjuvants.

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References