Immunological Responses in Human Papillomavirus 16 E6/E7-transgenic Mice to E7 Protein Correlate with the Presence of Skin Disease

I. H. Frazer, D. M. Leippe, L. A. Dunn, et al.

Cancer Res 1995;55:2635-2639. Published online June 1, 1995.

[CANCER RESEARCH 55, 2635–2639, June 15, 1995]

Immunological Responses in Human Papillomavirus 16 E6/E7-transgenic Mice to E7 Protein Correlate with the Presence of Skin Disease[1]

I. H. Frazer, D. M. Leippe, L. A. Dunn, A. Liem, R. W. Tindle, G. J. P. Fernando, W. C. Phelps, and P. F. Lambert[2]

McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin 53706 [D. M. L., A. L., P. F. L.]; Papillomavirus Research Unit, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia 4102 [I. H. F., L. A. D., R. W. T., G. J. P. F.]; and Division of Virology, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709 [W. C. P.]

ABSTRACT

The human papillomavirus (HPV) oncogenes, *E6* and *E7*, are believed to contribute to the development of cervical cancers in women infected with certain HPV genotypes, most notably HPV-16 and HPV-18. Given their expression in tumor tissue, *E6* and *E7* have been implicated as potential tumor-specific antigens. We have examined an HPV-16 E6- and E7-transgenic mouse lineage for immune responses to these viral oncoproteins. Mice in this lineage express the HPV-16 *E6* and *E7* genes in their skin and eyes, and on aging, these mice frequently develop squamous cell carcinomas and lenticular tumors. Young transgenic mice, which had measurable E7 protein in the eye but not in the skin, were immunologically naive to E7 protein. They mounted an immune response to E7 on immunization comparable to that of nontransgenic controls, suggesting a lack of immune tolerance to this protein. Older line 19 mice, which are susceptible to skin disease associated with transcription of the E6 and E7 open reading frames, had measurable E7 protein in their skin. These older transgenic mice spontaneously developed antibody responses to endogenous E7 protein, particularly in association with skin disease. Also detected in older mice were delayed-type hypersensitivity responses to E7. These finding parallel the humoral immune response to E7 protein in patients with HPV-associated cervical cancer and suggest that line 19 mice may provide a model for studying the immunobiology of HPV-associated cancers.

INTRODUCTION

A majority of cervical cancers contain HPV[3] DNA, and between 60 and 80% of these contain DNA from the HPV-16 genotype (1), commonly integrated within the cellular genome. In HPV DNA-positive cervical cancers, transcription of the E6 and E7 ORFs of HPV is observed, and a critical role for these viral gene products in cervical cancer can be inferred from the preservation in each cancer of these ORFs (2). Additional evidence for the role of E6 and E7 comes from the observation that expression of the E6 and E7 ORFs in primary human epithelial cell cultures facilitates their immortalization (3). Furthermore, mice transgenic for E6 and E7 develop epidermal hyperplasia (4, 5) and are at increased risk of epithelial tumor development (6).

That E6 and E7 are expressed in cervical cancers indicates that these viral gene products are potential tumor-specific antigens that might be the target of host-protective immune responses. The increased incidence of cervical cancer in immunosuppressed renal transplant recipients (7) and in patients with HIV-AIDS (8, 9) suggests that a protective host response occurs in immunocompetent patients. Antibody to the E7 ORF of HPV-16 has been demonstrated more frequently in patients with HPV-16-associated cancer than in control subjects not expected to be exposed to HPV-16 (10); this is observed particularly in patients with invasive HPV-16-associated tumors. A role of E7-directed immune responses in tumor protection has been raised by studies in which animals immunized against E7 acquired resistance to challenge with tumor cells expressing E7 (11). We have sought to assess the role of E7-specific immunity in a relevant mouse model that closely approximates the natural course of disease seen with human cervical cancer, and in which the papillomaviral oncogenes play a causative role in tumorigenesis. Several lines of mice transgenic for the HPV-16 E6 and E7 ORFs driven from the αA-crystallin promoter have been described recently (4). One of these lines of mice (αAcryHPV16E6/E7-line19) develops a skin pathology including hair loss, dermal thickening, papilloma development, and eventual skin cancer associated with increased levels of E6 and E7 mRNAs in the affected skin (12). This disease begins to appear at 3 months of age; by 1 year approximately 50% have abnormal skin. In the current study, we have characterized immunity to HPV-16 E7 protein in these mice and the relationship between any observed tolerance of, or immunity to, E7 and the development of the skin disease. As seen in cervical cancer patients, these mice were found to develop humoral and cellular immune responses against E7 protein, and this correlated with the presence of transgene-induced skin disease. These findings indicate that these HPV-16 transgenic mice may provide a useful model for studying the immunobiology of HPV-associated neoplasia.

MATERIALS AND METHODS

Mice. Nontransgenic FVB mice and αAcryHPV16E6/E7-line 19 mice derived from FVB mice and homozygous for a transgene that includes the E6 and E7 ORFs of HPV-16, driven by the αA-crystallin promoter, were held in standard American Association for Accreditation of Laboratory Animal Care approved mouse rooms under specific pathogen-free conditions.

Proteins and Peptides. A series of overlapping 18–26-mer peptides spanning the 98-amino acid predicted sequence of the E7 protein of HPV-16 (Fig. 1) were synthesized with the use of F-moc chemistry on an Applied Biosystems AB431 synthesizer (Applied Biosystems, Foster City, CA) as described previously (13). Synthetic peptides BT12D (DRAHYNIVTFCCKCDQAEPDAGIDGPAGEYMLD: single letter code) containing three B epitopes and a T-helper epitope from HPV-16 E7, GF110 (TRKSIRIQRGPDRAHYNIVTFCCKCD), including a HIV-1 gp120 B epitope and a T-helper epitope from HPV-16 E7, and GF22 (QDIVLHLEPQNEIPVDLL), which includes a B epitope from HPV-18 E7 protein, were synthesized similarly. Peptides were purified by HPLC and analyzed as described previously (13). An HPV-16 E7 GST fusion protein was prepared in *Escherichia coli* and purified as described (14). The bacterially produced fusion protein MS2-E7(15) was prepared from *E. coli* as described (16). Baculovirus-derived E7 protein was prepared as described and used as a crude cell lysate (17). A highly purified (>95%) preparation of bacterially produced HPV-16 E7 protein was prepared as described (18).

Received 1/19/95; accepted 4/18/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was in part supported by NIH Grants CA 57789, CA 22443, and CA 07175; the American Cancer Society; the National Health and Medical Research Council of Australia; and the Queensland Cancer Fund. I. H. F. was supported in part by a William Rudder Fellowship from the Queensland Cancer Fund. D. M. L. was supported in part by a fellowship from the Ladies Auxiliary to the Veterans of Foreign Wars of the United States. This work was performed while I. H. F. was on a sabbatical visit to the McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI.

[2] To whom requests for reprints should be addressed, at McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, 1400 University Avenue, Madison, WI 53706.

[3] The abbreviations used are: HPV, human papillomavirus; ORF, open reading frame; DTH, delayed-type hypersensitivity; i.d., intradermally.

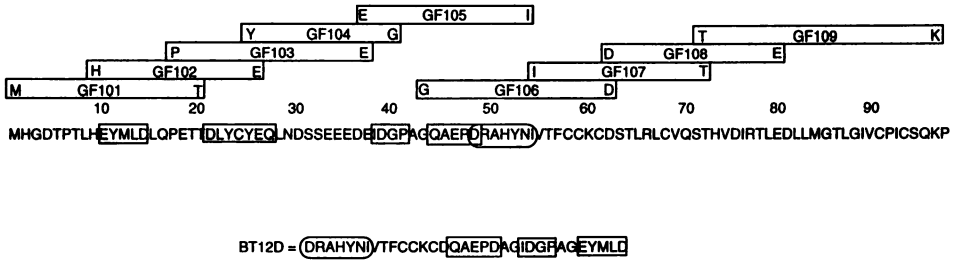

Fig. 1. Series of overlapping peptides GF101–GF109 that span the HPV16 E7 molecule. Known murine B epitopes are boxed; promiscuous T-helper epitope is ringed. The sequence of the synthetic peptide BT12D that contains three B epitopes and the T-helper epitope is shown below.

Immunization Protocol. Mice were immunized i.p. with an emulsion generated by mixing 50 mg of antigen, dissolved or suspended in 0.1 ml of 0.15 M NaCl with 0.1 ml of complete Freund's adjuvant, and boosted at days 14 and 28 with an equal amount of antigen in incomplete Freund's adjuvant. Sera were obtained from mice at day 42.

Assay of E7 Protein. Tissues for analysis were snap frozen in solid CO_2-ethanol and held at −70°C for analysis. Tissues were pulverized in a chilled mortar and pestle and assayed for HPV-16 E7 and HPV-18 E7 protein as a control, with the use of an ELISA capture assay standardized on HPV 16 E7 fusion protein essentially as described previously (14), except that extracted samples were boiled for 5 min with 0.1% SDS prior to analysis, which stabilizes the E7 protein (data not shown). Limits of E7 protein detection were established by performing the ELISA capture assay in parallel with serial dilutions of bacterially synthesized E7 fusion protein that had been purified and quantitated (see Ref. 14).

Antibody to E7 Protein and Peptides. An ELISA was used to measure antibody as described previously (16). In brief, peptides and proteins were dissolved in carbonate buffer (pH 9.4) and allowed to adsorb to 96-well plates. Plates were blocked with 2% BSA and exposed for 1 h to sera diluted 1:20 (or otherwise where stated) in PBS-2% BSA. Plates were washed extensively with PBS-0.1% gelatin-0.1% Tween 20, exposed to goat anti-mouse immunoglobulin or goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO), washed additionally, and developed with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid substrate. Absorbance measurements (414 and 620 nm) were assessed after 5–30 min of incubation with substrate on a Titertek Multiskan dual wavelength spectrophotometer (LabSystems, Helsinki, Finland).

DTH to E7 Protein. Mice were given 400 ng pertussigen (CSL, Melbourne, Australia) i.v. in 0.1 ml PBS (19) and immediately challenged i.d. in the left ear with 0.5–2.5 mg protein in 10 μl PBS. Ear thickness was assessed with the use of spring-loaded calipers (Mitutoyo 2046F) 48 and 96 h after challenge and was reported as the difference between the challenged and the control ear.

RESULTS

Induced Antibody Response to HPV-16 E7 Protein in Line 19 Mice. To analyze the potential of the αAcryHPV16E6/E7-line19 (herein referred to as line 19) mice to respond immunologically to E7 protein, young transgenic and nontransgenic animals were immunized with E7 protein and sera monitored for E7-specific antibodies. These transgenic mice developed E7-specific antibodies as did E7-immunized nontransgenic mice (Table 1). Furthermore, the transgenic and nontransgenic mice responded to similar linear epitopes from E7 (Fig. 2*A*), these epitopes being equivalent to those recognized by sera from E7-immunized mice of other haplotypes ($H\text{-}2^b$, $H\text{-}2^d$, $H\text{-}2^s$, $H\text{-}2^k$; the line 19 mice are in the FVB inbred background and are, therefore, $H\text{-}2^q$; Refs. 16, 20). Consistent with these observations, both the transgenic and nontransgenic mice developed E7-specific antibodies when immunized with an oligopeptide, BT12D, containing a known E7-specific T-helper epitope and the above noted E7-specific B cell epitopes (Table 1). Thus, the humoral responses of transgenic and nontransgenic mice were indistinguishable from each other.

E7 Protein Expression in the Skin of Line 19 Mice. Self-tolerance is argued to occur through the recognition of self-peptides expressed during postnatal development within immunosurveyed sites in the thymus (central or deletional tolerance) or other tissues (peripheral tolerance). Our E7 immunization studies suggested an absence of central and peripheral tolerance to E7 in the line 19 transgenic mice. To address whether this absence of tolerance correlated with an absence in the expression of E7 protein in immunosurveyed sites in young transgenic mice, we measured E7 protein levels in various organs of line 19 mice utilizing an ELISA-capture assay sensitive to 0.05 ng E7/mg cellular protein. No E7 protein was detected in any tissues from nontransgenic mice (data not shown). E7 protein was found in the eye of line 19 mice at an early age (Table 2). This was anticipated given that the transgene is under the control of the αA-crystallin promoter and is actively transcribed in the lens by day 17 in embryogenesis (4). Expression of E7 protein in the lens, however, would not be predicted to induce tolerance given that the lens is an immunoprivileged site. No E7 protein was detected in any other tissue of young line 19 mice, including the liver, spleen, brain, stomach, skin and, importantly, the thymus (Table 2; data not shown).

In our previous study, we demonstrated increased levels of E6 and E7 mRNAs in the abnormal skin and skin cancers in older line 19 mice (12). Skin samples from line 19 mice were, therefore, tested for E7 protein. Some older (>28 weeks old) mice were found to be positive for E7 protein in normal and diseased skin, as well as in a skin

Table 1 *Induced immune response to E7 proteins and peptides in young line 19 and FVB mice*

Immunogen	Mouse strain (no. of mice)	Mean absorbance units (±1 SEM)[a] for substrate antigen				
		BSA	E7	GST-E7	MS2-E7	BT12D
Saline	Line 19 (3)	0.002 ± 0.000	0.006 ± 0.001	0.018 ± 0.005	0.041 ± 0.015	0.002 ± 0.000
Saline	FVB (3)	0.003 ± 0.001	0.003 ± 0.001	0.005 ± 0.001	0.016 ± 0.003	0.002 ± 0.000
E7[b]	Line 19 (3)	0.001 ± 0.000	0.027 ± 0.004	0.055 ± 0.014	0.077 ± 0.020	0.015 ± 0.001
E7[b]	FVB (3)	0.005 ± 0.001	0.070 ± 0.037	0.095 ± 0.039	0.095 ± 0.038	0.066 ± 0.007
GST-E7	Line 19 (3)	0.001 ± 0.000	0.347 ± 0.022	0.481 ± 0.018	0.354 ± 0.028	0.119 ± 0.053
GST-E7	FVB (2)	0.001 ± 0.000	0.350 ± 0.026	0.372 ± 0.007	0.384 ± 0.068	0.189 ± 0.150
BT12D	Line 19 (4)	0.001 ± 0.000	0.194 ± 0.003	0.211 ± 0.007	0.172 ± 0.017	0.229 ± 0.012
BT12D	FVB (4)	0.001 ± 0.000	0.157 ± 0.023	0.162 ± 0.032	0.105 ± 0.023	0.222 ± 0.010
mAb α E7[c]		0.001	0.205	0.243	0.171	0.221

[a] Absorbance units (414–620 nm). Note the small but significant difference in reactivity of the saline/line 19 *versus* saline/FVB mouse sera to E7 substrates was not reproduced with additional bleeds from these same mice.
[b] Produced using a baculovirus vector.
[c] Control result with E7-specific mAb 6D (15) used at 1:1000.

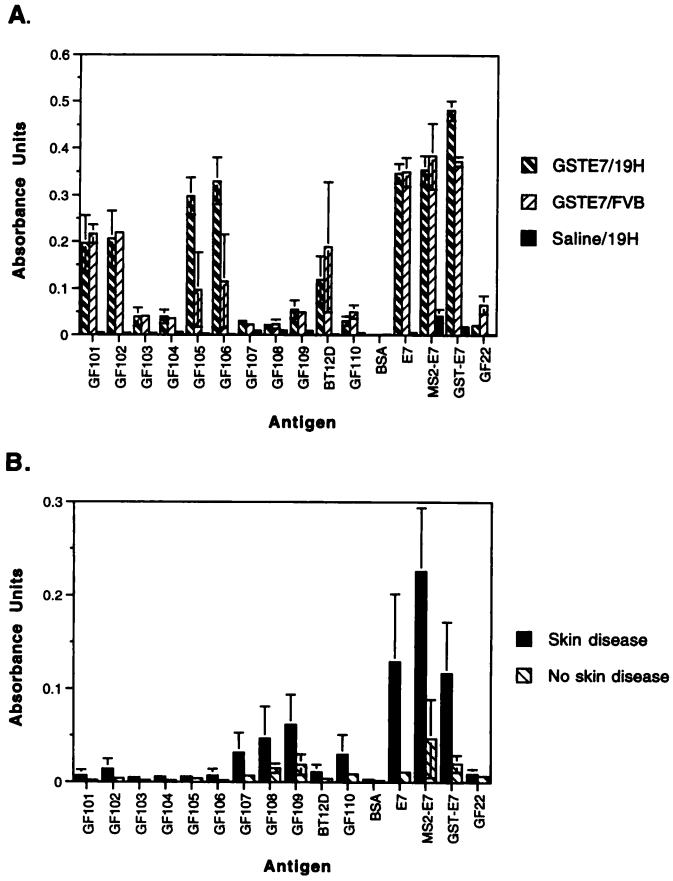

Fig. 2. Serum reactivity to HPV 16 E7 protein and peptides by ELISA. *A*, groups of three 6–8-week-old line 19H or FVB mice, as indicated, immunized with the stated antigen in complete Freund's adjuvant. *B*, groups of unimmunized 26–52-week-old line 19 mice with skin disease (n = 5), and littermate line 19 controls without skin disease (n = 3). Reactivity was shown to include antibody of IgG isotype with the use of isotype-specific antisera in the ELISA. *Columns*, mean; *bars*, SEM.

Table 2 *Immunoreactive E7 protein in the tissues of line 19 mice*

	Young mice (4–7 weeks)		Old mice (26–52 weeks)	
Tissue	n[a]	iE7[b]	n[a]	iE7[b]
Eye	1/1[c]	60	NT	
Eye tumor	NA		2/5	3.8–5.2
Normal skin	0/20		6/22	0.7–13.9
Diseased skin	NA		7/14	0.3–11.5
Skin tumors	NA		1/3	0.74

[a] *n*, number of positive samples/total number of samples tested; NA, not available; NT, not tested.

[b] Results are expressed as ng immunoreactive E7 protein (iE7) per mg cellular protein in positive samples. The lower detection limit of the assay is 0.05 ng iE7/mg cellular protein.

[c] Results from four eyes assayed together.

tumor. The highest levels of E7 observed in the skin of line 19 mice were 4-fold lower than levels in the CaSki cervical carcinoma cell line (50 ± 10 ng E7/mg cell protein). In contrast, skin samples from young (7–13-week-old) line 19 mice had undetectable E7 protein. Thus, expression of detectable levels of E7 protein was found to occur at a nonimmunoprivileged site specifically in older mice.

Spontaneous Antibody Response to E7 Protein and Peptides. Given the absence of tolerance in line 19 mice and the adult onset expression of E7 protein in the skin, we addressed whether these transgenic mice developed spontaneous immune responses to endogenously expressed E7 protein as a function of age. Humoral immune responses to E7 were assessed in young (4–7 weeks) and older (26–30 weeks) line 19 mice. E7-specific antibodies were found in some older line 19 mice and, particularly, in older line 19 mice with skin disease (Fig. 3; see also Fig. 2*B* for data on smaller groups of additional animals). This correlation between skin disease and serum antibody to E7 protein was significant (Table 3; P = 0.007) and reproducible among multiple independent ELISA assays on the same group of sera (data not shown). Thus, spontaneous humoral responses to E7 protein occur in the line 19 mice and correlate with the presence of skin disease.

The properties of the E7-reactive sera from these unimmunized line 19 mice differed from that of E7-immunized syngeneic mice in two respects: (*a*) the E7-reactive sera from unimmunized line 19 mice had antibody titers significantly lower than that in sera from E7 immunized mice. This is deduced from the reproducible difference in the strength of ELISA readouts against GST-E7 substrate for these two populations of animals as reported in Fig. 3 (mean absorbance units for the seven unimmunized older animals with skin disease = 0.180; note a similar mean value of 0.130 was found for a smaller group of 3 animals evaluated in Fig. 2*B*) *versus* Table 1 (mean absorbance units for E7-immunized line 19 mice = 0.481); and (*b*) the B-cell epitopes on E7 recognized by the E7-reactive, unimmunized line 19 mice mapped to the COOH terminus of E7 (Fig. 2*B*), and, therefore, differ from the NH_2 terminus-specific epitopes predominantly recognized in E7-immunized FVB mice (Fig. 2*A*).

Spontaneous Delayed-Type Hypersensitivity to E7 Protein. Ultimately, we are interested in understanding whether immune responses against E7, spontaneous or induced, can provide protection against the development of HPV-associated neoplasia. Such protective immune responses are thought to primarily rest in the cellular arm of the immune system. To test whether spontaneous cellular immune

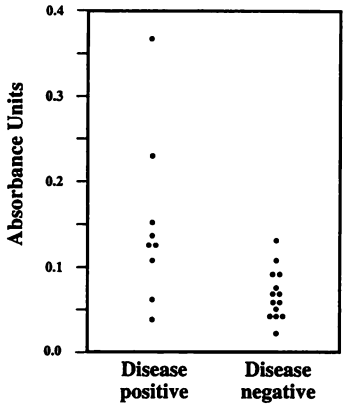

Fig. 3. Distribution of E7-specific ELISA readings for sera from older unimmunized line 19 mice. *Points*, absorbance measurements (414–620 nm) for sera reactivity to GST-E7 substrate. The mean background absorbance measurement for these sera (to BSA substrate) was 0.033 ± 0.009. Skin disease was scored by the presence of hair loss, with or without papillomatosis or carcinoma. See Table 3 for correlation between skin disease and E7 reactivity.

Table 3 *Associations between skin disease and serum antibody to E7 protein in older line 19 mice*[a]

	Serum E7 antibody	
Skin disease	Positive	Negative
Positive	7	2
Negative	2	12

[a] Number of animals in each group was determined based on the E7-reactivity data presented in Fig. 3. The correlation between E7 positivity of sera and skin disease had a calculated significance of P = 0.007 (Fischer's exact test). Sera were considered E7 reactive (serum E7 antibody positive) if they had ELISA readings that were 3-fold over background. The average of the ratio (E7-specific reactivity/BSA-specific reactivity) was 5.4 for the E7-reactive (positive) animals and 2.0 for the E7-nonreactive (negative) animals.

Table 4 *DTH to HPV-16 E7 protein in young and old line 19 transgenic mice and FVB controls*

Ear challenge	Δ Ear thickness (mm × 10^2 ± 1 SEM), (*n*)[a]		
	Old line 19	Young line 19	Old FVB
GST-E7	15 ± 4 (4)[b]	3 ± 1 (4)	3 ± 1 (4)
GST	2 ± 2 (3)	3 ± 1 (4)	6 ± 3 (3)
Saline	−1 ± 1 (4)	2 ± 2 (4)	0 ± 1 (4)

[a] Average of the difference between the challenged and control ear at day 4. *n*, number of mice/group. As positive controls, two E7-immunized line 19 mice gave DTH responses (Δ ear thickness) of 15.
[b] $P < 0.01$ compared with the control groups.

responses to endogenous E7 protein occur in our line 19 transgenic mice, experiments measuring DTH to HPV-16 E7 protein were performed, with the use of an ear challenge assay (Table 4). DTH is thought to represent antigen-specific cytokine-mediated inflammation, particularly involving Th1 type cytokines, in this case in response to endogenous E7 protein. DTH responses to E7 have been previously noted in mice that had received grafts of E7-positive cells (21). In our hands, positive DTH responses peaking at 48–96 h and specific to GST-E7 protein were observed in 3 of 4 older line 19 mice. Two of these DTH-positive animals also had skin disease and E7-specific serum antibodies. None of the younger mice, or the nontransgenic controls, developed DTH responses against E7. These results indicate that unimmunized older line 19 mice may develop a cellular immune response to E7 protein.

DISCUSSION

In this study we have demonstrated that a line of mice transgenic for HPV-16 E6 and E7 oncogenes develop spontaneous immune responses to E7 that correlate with the presence of skin disease. Unlike in E7-immunized mice challenged with transplantable tumors transduced to express E6 or E7 (11, 22, 23), the line 19 mice with spontaneous immune responses to E7 are not protected against the development of HPV-16-associated cancer. This finding parallels observations with cervical cancer patients in which there is a weak and inconsistent antibody response to E7 protein (24). We therefore believe that these mice may provide a valuable tool with which to study the immunobiology of HPV-16-associated cancer.

We were surprised initially to find an absence of tolerance to the E7 transgene in the line 19 mice. Determinants of the development of tolerance or immune responsiveness to transgenic proteins are uncertain. Deletional B- and/or T-cell tolerance is observed generally when the protein is expressed in a wide range of tissues from a strong constitutive promoter (25), whereas functional silencing of T or B cells (anergy; Ref. 26) or developing immunity (27, 28) has been observed in transgenic models in which there is tissue-specific expression of the transgene protein. In a number of transgenic mouse models, development of immunity or tolerance to the transgene product has been held to be a consequence of the level and/or timing of transgene expression (29, 30, 31). In this regard, the absence of tolerance to E7 in the line 19 mice correlated with an absence of detectable E7 protein expression in neonatal thymus (data not shown) and in all other tissues tested from young animals with the exception of the lens, an immunoprivileged site. The lack of E7 expression in immunosurveyed tissues during the time when deletional tolerance occurs provides one likely explanation for the absence of B- and T-cell tolerance in these animals.

We observed a correlation between skin disease and spontaneous immune responses to E7 protein in older line 19 mice (Table 3). The adult onset immune responses may reflect the observed increases in the levels of E7 protein expressed in immunosurveyed tissues (Table 2). However, the molecular mechanisms for this increased expression of E7 protein are not known at this time. Possible mechanisms include amplification of the transgenes, increased transcription of E7, or changes in the stability of E7 protein. Alternatively, the adult onset immune responses may represent an altered sensitivity of the immune system to presentation of E7-specific epitopes, perhaps mediated by cytokine induction resulting from skin wounding.

Despite the evidence for spontaneous humoral and cellular immune responses to E7 (Fig. 3; Tables 3 and 4), the line 19 mice develop squamous cell carcinomas in which the E7 oncoprotein is expressed (12). Thus, the spontaneous immune responses are not protective. It is clear from other studies that mice can develop protective immunity when E7 is presented optimally (11, 22, 23). The lack of protective immunity in the line 19 mice may reflect the nature of E7 presentation by the skin or tumor cells to the immune system. For instance, were E7 presented in line 19 mice by E7-positive keratinocytes (Table 2), which are nonprofessional antigen-presenting cells, this presentation may be suboptimal. We found the strength of the E7-specific spontaneous humoral immune responses in line 19 mice (Table 3) to be well below that seen in mice immunized with E7 (Table 1). This low level spontaneous immune response may not be sufficient to provide protective immunity. Additionally, the E7 reactive sera from the older, unimmunized line 19 animals predominantly recognized linear epitopes at the COOH terminus of E7 (GF107, GF108, and GF109; Fig. 2*B*), a region likewise recognized by sera from CBA ($H\text{-}2^k$) mice seeded with mouse L fibroblasts expressing the *E7* gene (20). In contrast, the E7-specific epitopes (residing within peptides GF101, GF102, GF105, and GF106) strongly recognized by the E7-immunized transgenic and nontransgenic mice (Fig. 2*A*) lie in the NH_2 terminus of E7 protein. These findings suggest differential processing or presentation of E7 protein by professional *versus* nonprofessional antigen-presenting cells, and this difference could contribute to the absence of protective immunity. A better understanding of the immunobiology of the line 19 mice may provide insight into how the immune system, when faced with the suboptimal presentation of papillomaviral antigens, can be induced to generate protective immunity against HPV-associated cancer.

REFERENCES

1. Munoz, N., and Bosch, F. X. HPV and cervical neoplasia: review of case-control and cohort studies. *In:* N. Munoz, F. X. Bosch, K. V. Shah, and A. Meheus (eds.), The epidemiology of human papillomavirus and cervical cancer, pp. 251–261, IARC Scientific Pub. No. 119. Lyon, France: IARC, 1992.
2. Schwarz, E., Freese, U. K., Gissmann, L., Mayer, W., Roggenbuck, B., Stremlau, A., and Zur Hausen, H. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature (Lond.), *314:* 111–114, 1985.
3. McDougall, J. K. Immortalization and transformation of human cells by human papillomavirus. Curr. Top. Microbiol. Immunol., *186:* 101–119, 1994.
4. Griep, A. E., Herber, R., Jeon, S., Lohse, J. K., Dubielzig, R. R., and Lambert, P. F. Tumorigenicity by human papillomavirus type 16 E6 and E7 in transgenic mice correlates with alterations in epithelial cell growth and differentiation. J. Virol., *67:* 1373–1384, 1993.
5. Arbeit, J. M., Münger, K., Howley, P. M., and Hanahan, D. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J. Virol., *68:* 4358–4368, 1994.
6. Griep, A. E., and Lambert, P. F. Role of papillomavirus oncogenes in human cervical cancer: transgenic animal studies. Proc. Soc. Exp. Biol. Med., *206:* 24–34, 1994.
7. Shiel, A. G. R. Cancer report. *In:* A. P. S. Disney (ed.), ANZDATA Report 1991, pp. 100–108, Adelaide, South Australia: Australia and New Zealand Dialysis and Transplant Registry, 1991.
8. Williams, A. B., Darragh, T. M., Vranizan, K., Ochia, C., Moss, A. R., and Palefsky, J. M. Anal and cervical human papillomavirus infection and risk of anal and cervical epithelial abnormalities in human immunodeficiency virus-infected women. Obstet. Gynecol., *83:* 205–211, 1994.
9. Northfelt, D. W. Cervical and anal neoplasia and HPV infection in persons with HIV infection. Oncology, *8:* 33–37, 1994.
10. Jochmus-Kudielka, I., Schneider, A., Braun, R., Kimmig, R., Koldovsky, U., Schneweis, K. E., Seedorf, K., and Gissmann, L. Antibodies against the human papillomavirus type 16 early proteins in human sera: correlation of anti-E7 reactivity with cervical cancer. J. Natl. Cancer Inst., *81:* 1698–1704, 1989.

11. Feltkamp, M. C. W., Smits, H. L., Vierboom, M. P. M., Minnaar, R. P., De Jongh, B. M., Wouter Drijfhout, J., Ter Schegget, J., Melief, C. J. M., and Kast, W. M. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. Eur. J. Immunol., *23:* 2242–2249, 1993.
12. Lambert, P. F., Pan, H., Pitot, H. C., Liem, A., Jackson, M., and Griep, A. E. Epidermal cancer associated with expression of human papillomavirus type 16 *E6* and *E7* oncogenes in the skin of transgenic mice. Proc. Natl. Acad. Sci. USA, *90:* 5583–5587, 1993.
13. Tindle, R. W., Fernando, G. J. P., Sterling, J. C., and Frazer, I. H. A "public" T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes. Proc. Natl. Acad. Sci. USA, *88:* 5887–5891, 1991.
14. Selvey, L. A., Dunn, L. A., Murray, B., Tindle, R. W., and Frazer, I. H. An ELISA capture assay for the E7 transforming proteins of HPV16 and HPV18. J. Virol. Methods, *37:* 119–128, 1992.
15. Seedorf, K., Oltersdorf, T., Krammer, G., and Rowenkamp, W. Identification of early proteins of the human papillomaviruses type 16 (HPV 16) and type 18 (HPV 18) in cervical carcinoma cells. EMBO J., *6:* 139–144, 1987.
16. Tindle, R. W., Smith, J. A., Geysen, H. M., Selvey, L. A., and Frazer, I. H. Identification of B epitopes in human papillomavirus type 16 E7 open reading frame protein. J. Gen. Virol., *71:* 1347–1354, 1990.
17. Park, D. S., Selvey, L. A., Kelsall, S. R., and Frazer, I. H. Human Papillomavirus type 16 E6, E7 and L1 and type 18 E7 proteins produced by recombinant baculoviruses. J. Virol. Methods, *45:* 303–318, 1993.
18. Pahel, G., Aulabaugh, A., Short, S. A., Barnes, J. A., Painter, G. R., Ray, P., and Phelps, W. C. Structural and functional characterization of the HPV16 E7 protein expressed in bacteria. J. Biol. Chem., *268:* 26018–26025, 1993.
19. Sewell, W. A., Munoz, J. J., and Vadas, M. A. Enhancement of the intensity, persistence and passive transfer of delayed type hypersensitivity lesions by pertussigen in mice. J. Exp. Med., *157:* 2087–2096, 1983.
20. Comerford, S. A., McCance, D. J., Dougan, G., and Tite, J. P. Identification of T- and B-cell epitopes of the E7 protein of human papillomavirus type 16. J. Virol., *65:* 4681–4690, 1991.
21. Chambers, M. A., Wei, Z., Coleman, N., Nash, A. A., and Stanley, M. A. "Natural" presentation of human papillomavirus type-16 E7 protein to immunocompetent mice results in antigen-specific sensitization or sustained unresponsiveness. Eur. J. Immunol., *24:* 738–745, 1994.
22. Chen, L., Mizuno, M. T., Singhal, M. C., Hu, S-L., Galloway, D. A., Hellström, I., and Hellström, K. E. Induction of cytotoxic T lymphocytes specific for a syngeneic tumor expressing the E6 oncoprotein of human papillomavirus type 16. J. Immunol., *148:* 2617–2621, 1992.
23. Chen, L., Thomas, E. K., Hu, S., Hellström, I., and Hellström, K. E. Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection antigen. Proc. Natl. Acad. Sci. USA, *88:* 110–114, 1991.
24. Tindle, R. W., and Frazer, I. H. Immune response to human papillomaviruses and the prospects for human papillomavirus-specific immunization. Curr. Top. Microbiol. Immunol., *186:* 217–253, 1994.
25. Ramsdell, F., and Fowlkes, B. J. Clonal deletion *versus* clonal anergy: the role of the thymus in inducing self tolerance. Science (Washington DC), *248:* 1342–1348, 1990.
26. Miller, J. F. A. P., and Morahan, G. Peripheral T cell tolerance. Annu. Rev. Immunol., *10:* 51–69, 1992.
27. Ohashi, P. S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C. T., Odermatt, B., Malissen, B., Zinkernagel, R. M., and Hengartner, H. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell, *65:* 305–317, 1991.
28. Milich, D. R., McLachlan, A., Raney, A. K., Houghten, R., Thornton, G. B., Maruyama, T., Hughes, J. L., and Jones, J. E. Autoantibody production in hepatitis B e antigen transgenic mice elicited with a self T-cell peptide and inhibited with nonself peptides. Proc. Natl. Acad. Sci. USA, *88:* 4348–4352, 1991.
29. Hasegawa, T., Isobe, K., Nakashima, I., and Shimokata, K. Quantitative analysis of antigen for the induction of tolerance in carcinoembryonic antigen transgenic mice. Immunology, *77:* 577–581, 1992.
30. Abramczuk, J. W., Kearns Jonker, M., Monell Torrens, E., Wohlenberg, C., and Notkins, A. L. Transgenic mouse lines that are immunologically tolerant and nontolerant to herpes simplex virus glycoprotein D. J. Virol., *65:* 6124–6128, 1991.
31. Skowronski, J., Jolicoeur, C., Alpert, S., and Hanahan, D. Determinants of the B-cell response against a transgenic autoantigen. Proc. Natl. Acad. Sci. USA, *87:* 7487–7491, 1990.