Immune Response During Therapy With Cisplatin or Radiation for Human Papillomavirus–Related Head and Neck Cancer

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Background: Human papillomavirus (HPV) is the most identifiable cause of head and neck squamous cell cancer (HNSCC). Compared with HPV-negative HNSCC, HPV-positive HNSCC presents at an advanced stage but with significantly better survival. We created a syngeneic mouse model of HPV-positive and HPV-negative HNSCC by transforming mouse primary tonsil epithelial cells with either HPV oncogenes or a nonantigenic RNA interference strategy that affects similar oncogenic pathways.

Objectives: To examine the effect of radiation therapy on HPV-positive and HPV-negative tumors in immune-competent and immune-incompetent mice and to examine responses in human cancer cell lines.

Design: Prospective in vivo murine model.

Main Outcome Measures: Survival and tumor growth.

Results: For human and murine transformed cell lines, HPV-positive cells were more resistant to radiation and cisplatin therapy compared with HPV-negative cells. In vivo, HPV-positive tumors were more sensitive to radiation, with complete clearance at 20 Gy, compared with their HPV-negative counterparts, which showed persistent growth. Cisplatin in vivo cleared HPV-positive tumors but not HPV-negative tumors. However, neither radiation or cisplatin therapy cured immune-incompetent mice. Adoptive transfer of wild-type immune cells into immune-incompetent mice restored HPV-positive tumor clearance with cisplatin therapy.

Conclusions: The HPV-positive tumors are not more curable based on increased epithelial sensitivity to cisplatin or radiation therapy. Instead, radiation and cisplatin induce an immune response to this antigenic cancer. The implications of these results may lead to novel therapies that enhance tumor eradication for HPV-positive cancers.


QUAMOUS CELL CANCERS (SCCs) of the tonsillar region can be separated on a molecular level into cancers that are and are not associated with human papillomavirus (HPV). Previous findings have confirmed that HPV-associated cancers defy the previously held paradigm in which the stage at diagnosis correlates with prognosis. Advanced-stage HPV-associated cancers have a higher cure rate than do HPV-negative cancers. The presence or absence of viral oncogenes is a distinguishing feature that allows for molecular examination of this difference in therapeutic response.

The HPV status of tonsillar cancers is not used as a routine indicator to guide the choice among the various therapeutic options. Typical treatment involves surgical excision of early cancers and/or concurrent platinum-based chemotherapy (cisplatin or carboplatin) and fractionated radiation therapy. Radiation therapy has developed as a successful treatment option for head and neck SCC (HNSCC). Current therapies provide fractionated doses of radiation, with a significant proportion of individuals reaching complete remission. Radiation therapy for stages III and IV oropharyngeal cancer offers reported 5-year survival of 10% to 20%. Cisplatin therapy concurrent with radiation therapy improves clinical response rates and survival rates for advanced-stage cancers. Cisplatin acts by reacting with guanine and forming intrachromosomal and interchromosomal bonds. Formation of these bonds overwhelms DNA repair, thus inducing irreversible damage that leads to cell death. Rapidly dividing tumor cells are, thus, more sensitive to this DNA damage. The combination of radiation and cisplatin synergize to produce irreversible damage.

The improved survival of HPV-positive patients could be owing to multiple fac-
tors. For example, it is possible that HPV-positive cells are intrinsically more sensitive to these standard therapies and, thus, respond better to treatment. Recent study findings\(^5\,8\) suggest that HPV-related cancers actually display enhanced sensitivity to concurrent chemoradiation therapy. Another possibility is that HPV-positive tumors uniquely express foreign (viral) proteins and that an immune response is induced during therapy that helps clear tumors and prevent recurrence.

To better understand why HPV-associated cancers more readily respond to therapy, we developed in vitro and in vivo methods using human cancer cell lines and primary tonsillar keratinocytes (mouse and human) transformed with HPV oncogenes.\(^9\,10\) These methods allow us to better understand responses to common therapies used for this cancer type. In this study, we examine the response of HPV-positive and HPV-negative cancer cells to these therapies in vitro and in a mouse model that replicates many aspects of human disease. The findings demonstrate that an immune response augments the known antitumor action of combined cisplatin and radiation therapy. This immune response is required to clear established cancer. These findings have implications for (1) other antigenic tumors (such as Epstein-Barr virus–related nasopharyngeal carcinoma) that are treated with platinum-based chemotherapy and radiation, (2) the development of further adjuvant therapies that could potentially enhance this immune response, and (3) understanding the poor response to therapy in individuals with suppressed immunity.

**METHODS**

### CELL CULTURE

Normal mouse tonsil epithelial cells (MTECs) were isolated from the epithelium overlying C57BL/6 mouse and human tonsil tissue as previously described.\(^9\,11\) The E6E7/Ras and shPPTN13/Ras MTEC lines, generated from normal MTECs as previously described, were maintained in E-media.\(^9\,10\) The HNSCC tumor lines UMSCC-1, -19, and -84 (HPV negative) and UMSCC-47 and UPCI-SCC90 (HPV positive) were maintained in a combination of Dulbecco modified Eagle medium, 10% fetal calf serum, and penicillin-streptomycin, 1%.

### CLONOGENIC ASSAY

Cell lines were seeded on 60-mm plates (500 cells per plate) in triplicate at each concentration. Cisplatin (0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 µg/mL in dimethyl sulfoxide) was added 5 hours later. Cisplatin was removed along with a medium change 24 hours later. In experiments in which cells were treated with combined cisplatin and radiation, plates were irradiated (0, 2.5, 5, 8, and 16 Gy) 2 hours after the addition of cisplatin. Cisplatin doses for combined cisplatin and radiation therapy were 0 and 0.25 µg/mL. Head and neck cancer cells were treated only with radiation (0, 2, 4, 6, and 8 Gy). Cells were allowed to grow until colonies of untreated controls reached 50 or more cells (10-14 days). Plates were fixed with 70% ethanol and were stained with Coomassie blue; colonies with more than 15 cells were counted. Each experimental condition was repeated in triplicate (3 plates per condition), and the surviving fraction was calculated using the following formula: (colonies counted)/(cells plated × plating efficiency/100). Plating efficiency was calculated as the number of cells present 24 hours after plating divided by the number of cells plated at time zero. The experiment was repeated, and the average of the experiments is plotted with standard errors. Statistical analysis of the cisplatin in vitro assay was performed using the Mann-Whitney test.

### IN VIVO ASSAY

In vivo growth was assayed using previously described techniques.\(^11\) All experiments were performed in accord with institutional and national guidelines and regulations; the protocol was approved by the animal care committees at the University of Iowa and Sanford Research. Briefly, using an 18-gauge needle, C57BL/6 mice (immune competent) and B6.129S7-Rag1<sup>−/−</sup> mice (RAG-1–immunocompetent, lacking B and T cells) were injected with 1 × 10<sup>6</sup> cells in the subcutaneous tissue of the flank (6 mice per treatment condition; the experiment was repeated, and the results were averaged). The mice were treated with cisplatin dissolved in an isotonic sodium chloride solution (5, 10, or 20 mg/m<sup>2</sup>) and administered intraperitoneally weekly for 3 weeks. Treatment with cisplatin began 1 week after tumor cell injection, unless otherwise specified. For animals treated with radiation alone, 0, 8, 16, 24, or 32 Gy of radiation was administered to the tumor site, with lead shielding to the rest of the body, at the University of Iowa animal radiation facility (cesium source). Animals were euthanized when the tumor size was greater than 20 mm in its greatest dimension or when the animal was substantially emaciated. Mice were considered tumor free when they showed no evidence of tumor after 3 months. Survival graphs were calculated by standardizing the date of death for a 2-cm tumor. Statistical analysis for the survival graphs was performed using the log-rank test, with α = 0.01.

### IMMUNIZATION

The development of adenovirus with no reporter gene (Ad-Empty) and adenovirus expressing HPV-16 E6 and E7 oncoproteins (Ad-E6/E7) was previously described.\(^12\) Adenovirus (10<sup>7</sup> particles) suspended in 50 µL of phosphate-buffered saline solution was delivered to the nasal passages of anesthetized C57BL/6 mice either 7 or 14 days before undergoing radiation therapy alone (6 mice per treatment group; the results were averaged across 2 experiments). The mice were treated with 20 Gy. For combined cisplatin and radiation therapy plus vaccine experiments, the same dose of virus was delivered 7 days before treatment, with cisplatin at 20 mg/m<sup>2</sup> and radiation at 8 Gy given once a week for 3 weeks, to a total dose of 24 Gy.

### ISOLATION OF SPLENOCYTES

The mice were euthanized, and their spleens were harvested through an abdominal incision. The spleens were homogenized in a phosphate-buffered saline solution. The resulting phosphate-buffered saline–splenocyte mixture was spun at 0.2 relative centrifugal force for 5 minutes, supernatant was aspirated and discarded, and the pellet was resuspended in 10 mL of ACK-lysing buffer (ammonium chloride, 8.29 g/L; potassium bicarbonate, 1.00 g/L; and disodium EDTA–2H<sub>2</sub>O, 0.0372 g/L). Four milliliters of a phosphate-buffered saline solution (pH, mean [SD], 7.4 [0.2]) was used to neutralize the ACK-lysing buffer, and the resulting solution was flash spun to isolate larger debris. The supernatant was poured into a new tube and was spun at 0.2 relative centrifugal force for 5 minutes. The resulting supernatant was discarded, and the pellet composed of splenocytes was resuspended in 10 mL of spleenocyte medium (5% fetal calf serum, penicillin [1 U/mL]–
ADOPTIVE TRANSFER

C57BL/6 RAG-1 mice were anesthetized using ketamine, 0.1 mL, intraperitoneally, and then 1.6 × 10⁶ splenocytes were injected retro-orbitally into each mouse. Seven days after splenocyte transfer, the C57BL/6 RAG-1 mice were injected with 1 × 10⁸ E6E7/Ras MTECs and then were treated with cisplatin as described in the “In Vivo Assay” subsection of the “Methods” section (6 mice per treatment group; the results were averaged across 2 experiments). Seven weeks after initiation of the experiment, the C57BL/6 RAG-1 mice were euthanized, their splenocytes were isolated using the aforementioned protocol, and splenocyte populations of CD4, CD8, CD40, and CD25 were examined using flow cytometry.

IN VITRO CLONOGENIC SURVIVAL

It is possible that the survival advantage of patients with HPV-positive HNSCC treated with chemoradiotherapy is due to an inherent radiosensitivity of HPV-positive tumor cells compared with HPV-negative tumor cells. To test this hypothesis, we completed clonogenic survival assays with increasing radiation doses in vitro for several HPV-positive and HPV-negative HNSCC lines. The HPV-positive cell lines (UMSCC-47 and UPCI-SCC90) were slightly more resistant to increasing doses of radiation compared with the HPV-negative cell lines (UMSCC-1, UMSCC-19, and UMSCC-84) (Figure 1A). In preparation for anticipated in vivo studies with mice, clonogenic survival assays were performed with HPV-positive and HPV-negative transformed MTECs treated with graded radiation doses. The transformation and characterization of these MTECs have been previously described.10,11 Similar to the human cell lines, the HPV-positive MTECs exhibited greater survival in vitro than did the HPV-negative MTECs (Figure 1B). These findings are contrary to the hypothesis of an inherent sensitivity of HPV cells to radiation therapy.

RADIATION RESPONSE IN VIVO

The in vitro results showed relative resistance to radiation therapy for HPV-positive cells. Next, we used the immune-competent mouse model and examined response to the HPV-positive and HPV-negative cell lines in vivo (Figure 2). In matched mice, HPV-positive and HPV-negative cells demonstrated a dose response to radiation; however, the HPV-positive tumors had sustained complete responses in 80% of mice receiving more than 20 Gy of radiation. On the other hand, the HPV-negative cell lines showed only a partial response to therapy, and only 1 mouse that received the highest dose of radiation (32 Gy) remained disease free. Therefore, the HPV-positive tumors in mice are more easily cleared in vivo.

Although many factors could result in a survival difference in vitro vs in vivo, we hypothesized that an immune response may be enhancing clearance of the HPV-positive cells in vivo. To understand whether an immune response played a role in clearance of the HPV-positive tumors, we repeated the same assay in C57BL/6 RAG-1 mice. The RAG-1 mice are inbred mice that are genetically nearly identical to C57BL/6 mice except that they have a mutated RAG-1 gene, which is essential for the development of functional T and B cells.13 A dose-dependent radiation response was again seen, but complete remission was attainable in only 1 mouse at the highest dose of radiation (Figure 2C). Unlike immune-competent mice (C57BL/6 mice), RAG-1 mice rarely cleared the HPV-positive tumors after undergoing radiation. The survival difference (80%) between wild-type and RAG-1 mice with HPV-positive implanted and treated with equivalent doses of radiation (0, 8, 16, and 24 Gy) was significant (P = .002, .007, .006, and .003, respectively). Thus, the immune system is important in clearing HPV-positive cells treated with radiation, and the presence of the HPV-positive oncogenes E6 and E7 is necessary for the observed survival advantage.
RADIATION EFFECT IN VIVO ON LARGER-VOLUME TUMORS

Unlike the mice we tested in the experiments described herein, most patients present with tumors well past the approximate 10-million-cell stage, and their tumors have been present for weeks to months. To test whether a single dose of radiation was sufficient to induce clearance in the treatment of established HPV-positive tumor volume of approximately 1000 mm³, mice were irradiated with 20 Gy. Compared with no treatment (control), radiation therapy limited tumor growth and resulted in prolonged survival time (Figure 3). However, all of the mice had tumors at the end of the experiment, and all of the mice died as a result of persistent disease. Given the role of the immune system noted previously herein, we postulated that enhancing the HPV-specific immune response may help improve response during radiation therapy. We previously developed an adenoviral vaccine that targets the HPV oncoproteins E6 and E7 and is effective at inducing clearance of HPV-positive tumor cells expressing E6 and E7 if given before tumor implantation.12 We used the Ad-E6/E7 vaccine or an adenovirus control (AdEmpty) to test whether an immune response to the E6 and E7 HPV oncoproteins would improve radiation response to HPV-positive tumors. Compared with vector controls, priming the mice with Ad-E6/E7 7 or 14 days before undergoing radiation therapy resulted in slower tumor growth and prolonged survival but did not result in tumor eradication (0% survival in all groups) (Figure 3). In this HPV-16 murine tumor system, a single treatment with 20 Gy of radiation alone cannot cure established tumors, and enhancing the immune response by vaccination against viral oncoproteins enhances response but is insufficient to induce cure.

RESPONSE OF HPV-POSITIVE MTECs AND HPV-NEGATIVE MTECs TO CISPLATIN IN VITRO

The most common chemotherapy agent for treating HPV-related oropharyngeal cancers is cisplatin.14 Examination of HPV-positive and HPV-negative head and neck cancer cell lines for in vitro sensitivity to cisplatin showed no significant difference based on HPV status (data not shown). To determine the cellular toxic effects of cisplatin therapy on HPV-positive and HPV-negative tumorigenic MTECs, we performed clonogenic assays using these 2 cell lines. A dose response to increasing concentrations of cisplatin for each cell line is shown in Figure 4. The HPV-positive cells are more resistant (63%, P < .02) to cisplatin compared with the HPV-negative cell lines. Based on these data, we predicted that the HPV-positive tumors would be more resistant to cisplatin therapy in vivo.

SENSITIVITY OF HPV-POSITIVE TUMORS TO CISPLATIN IN VIVO

To test cisplatin sensitivity in vivo, immune-competent mice were injected with either HPV-positive or HPV-negative tumorigenic MTECs. We began treatment with cisplatin after 7 days, when tumors were barely palpable. Figure 5A and B show the weekly average tu-
In vivo treatment with cisplatin completely cleared all 6 HPV-positive tumors, but there was a difference at the maximum dose used. Cisplatin treatment at 20 mg/m² slowed HPV-negative tumor growth and prolonged survival but did not cure a single mouse. On the other hand, the same dose of cisplatin completely cleared all 6 HPV-positive tumors. One mouse developed a recurrence 5 weeks after the last cisplatin treatment, which brought overall survival (0%) was not different from that of RAG-1 mice treated with cisplatin alone (data not shown).

To ascertain that an adoptive transfer occurred in these mice, we removed their spleens after the experiment reached its end point (tumor size ≥ 20 mm). The RAG-1 splenocyte population was examined using flow cytometry for levels of CD8, CD4, CD40, and CD25 cells, which were then compared with splenocyte populations from naïve RAG-1 and C57BL/6 mice. All adoptively transferred RAG-1 mice contained donor CD8-, CD4-, CD40-, and CD25-expressing cells, but the levels were lower than those for wild-type C57BL/6 mice (data not shown). These data further support the finding that an immune response aids tumor clearance during cisplatin therapy, but they also suggest that the transfer of cells did not completely reconstitute a fully functional immune response, which is required for total tumor clearance.
CISPLATIN THERAPY OF LARGE TUMORS

The HPV-positive tumors cured with cisplatin therapy in the in vivo experiment represented relatively small-volume early disease. To better understand whether cisplatin therapy and the immune response are sufficient to also clear large-volume established disease, C57BL/6 mice were injected with HPV-positive cells. The tumors were allowed to grow for 1 or 3 weeks (average tumor volume, 1000 mm$^3$) before beginning the cisplatin regimen (20 mg/m$^2$ weekly for 3 weeks). Cisplatin treatment slowed progression of tumor growth. However, if tumors were larger at the initiation of therapy, cisplatin was insufficient to induce a complete response or clearance in wild-type mice (Figure 7).

CONCURRENT CISPLATIN AND RADIATION THERAPY OF BULKY ESTABLISHED DISEASE

Combining cisplatin chemotherapy with radiation therapy has been shown in multiple studies to increase complete response and survival. In vitro studies have shown that this enhanced response is at least in part due to a synergistic action of tumor toxicity for the combined therapies. Because our data show that an immune response is as important in clearing HPV-positive tumors in mice treated with radiation therapy or chemotherapy alone, we examined the effect that the immune response played in clearing HPV-positive cancers during concurrent radiation therapy and chemotherapy in vitro. Figure 8 shows that cisplatin enhanced cell toxic effects for HPV-positive and HPV-negative cells. Differences in responses between the cell lines to concurrent therapy are not statistically significant.

We next tested whether an immune response was required to clear well-established HPV-positive disease. In these experiments, we let the tumors grow to 1 cm in greatest dimension before administering treatment. Previous experiments treating larger tumors with either cisplatin (20 mg/m$^2$ every week for 3 weeks) or radiation alone (a single dose of 24 Gy) produced a partial response but did not lead to a complete response or survival for any mice. Therefore, to test response to concurrent therapy, cisplatin was delivered in weekly doses (20 mg/m$^2$ every week for 3 weeks) and radiation (8 Gy every week for 3 weeks) was given on the infusion days. To compare responses in immune-competent and immune-incompetent animals, we delivered the same therapies to wild-type and RAG-1 mice. Comparison of RAG-1 mice receiving no treatment vs chemoradiation in Figure 9 shows that concurrent chemoradiation produces a partial response in the absence of an immune response (RAG-1 AdEmpty vs RAG-1 no treatment). A complete response was seen in 50% of immune-competent
mice treated with chemoradiation therapy (wild-type AdEmpty). The addition of Ad-E6/E7 14 days after tumor injection combined with chemoradiation cleared tumors in 90% compared with 50% clearance with AdEmpty. We concluded that the vaccine plus concurrent cisplatin and radiation therapy improves tumor killing and prolongs survival.

**COMMENT**

Tonsillar cancer, although histologically similar, is caused by random mutations (HPV negative) or by viral oncogenes plus limited random mutations (HPV positive). This dichotomy allows exploration of targeted therapy to viral oncogenes in HPV-positive head and neck cancer with treatment based on a molecular test for HPV. Typing of HPV can already provide prognostic information for patients. Data from this study examining response to current standard therapies allow us to draw several conclusions: (1) HPV-positive tumors are not more sensitive to radiation therapy in vitro; (2) in addition to direct cell toxic effects, an immune response helps clear HPV-positive cells that are directly irradiated; (3) HPV-positive cells are not more sensitive to cisplatin therapy; (4) similar to radiation therapy, cisplatin has a direct toxic effect and the immune response required for clearing HPV-positive cells; and (5) therapies that improve an anti-HPV immune response will likely provide improved treatment outcomes.

**MECHANISMS OF HPV TUMOR RESISTANCE TO RADIATION THERAPY**

Culture of tumor cells in vitro is, by definition, an artificial environment. Previous studies have shown a correlation between the radiosensitivity of cells in vitro and in vivo tumor growth. However, the human cell lines were placed into a xenograft mouse model with inherent immune reactivity limitations. More recently, the radiosen-
The immune system has a critical role in response during therapy. It has become increasingly clear for many cancers that the immune system plays a significant role in response during therapy. Although cisplatin is a known immunosuppressive agent, it does not suppress the function of the vaccine. This agrees with the current literature that cisplatin does not impair the effectiveness of CRT/E7 DNA vaccine against E7-expressing tumors. Recent evidence suggested that certain therapies not only have the direct effect of killing tumor cells but that the manner of cell death induced by the cytotoxic drug is important for inducing an immune response. Radiation and oxaliplatin (a drug similar to cisplatin) have previously been shown to induce an immune response during therapy in experimentally tested models. The HPV-positive tumors contain foreign viral proteins. The presence of viral oncoproteins in the cell could trigger an HPV-specific immune response. For example, in an antigenic model of fibrosarcoma and colon carcinoma, radiation has been shown to cause calreticulin to transit to the cell surface. The radiation-induced enhancement of calreticulin has been shown to be necessary to develop an antitumor response. Similarly, the mechanism for oxaliplatin has been shown to have direct cellular toxic effects and enhancement of tumor-mediated cell killing. Therefore, it is possible that antigen presentation, immune response, and E6 and E7 clearance are enhanced through radiation-mediated tumor damage. This enhanced immune clearance of cells with E6 and E7 antigens could explain the improved survival and decreased recurrence seen in HPV-positive HNSCC treated with cisplatin and radiation. The importance of the immune response in HPV-positive cells is shown in the durable clearance of tumor in immune-competent mice but not...
in RAG-1 mice. We cannot re-establish HPV-positive tumor cells in mice that clear their tumors during chemoradiation (data not shown). Such a finding would coincide with the finding that people with HPV-positive HNSCC have fewer recurrences after treatment. Although the mouse provides a useful model to complete the basic scientific work, future work needs to confirm these findings in humans and begin to determine mechanisms for the HPV-induced immune clearance.

**IMPLICATIONS FOR THERAPY**

Current treatment of HNSCC of the tonsillar region involves combined treatment with surgery, chemotherapy, and radiation therapy. Treatment is not stratified based on HPV status, and many tumors go without HPV tissue typing. The finding that mouse HPV-positive tumors are more sensitive to platinum-based chemotherapy (cisplatin) and radiation than are HPV-negative tumors correlates with the improved survival noted in previous clinical trials. The present mouse data and the review of previous human studies suggest that it would be wise to further investigate whether primary treatment with concurrent cisplatin and radiation should be a mainstay in the treatment of all HPV-positive cancers. The fact that neither therapy alone cleared the larger bulky tumors supports the finding that survival is improved in advanced-stage oropharyngeal cancers with concurrent delivery of cisplatin and radiation. It is possible that surgical removal of the tumor before administering postoperative chemoradiation therapy may further increase survival. Such a study will be examined in future experiments using the present mouse model and also could be applied to a correctly designed human study.

The present data also suggest that individuals who have an intact immune response will do better during therapy. Patients undergoing renal transplantation are at increased risk for viral-related, including HPV, cancers. In future work, it is important to also examine whether patients who are immunosuppressed from human immunodeficiency virus, organ transplantation, or systemic disease (such as severe cachexia) may have a more limited response to treatment of HPV-positive HNSCC with combined cisplatin and radiation. It would also be important when evaluating any adjuvant chemotherapy. If this therapy disrupts immune function, it actually may make treatment response worse.

Patients do not typically present with microscopic or small tumor burden. Although we achieved clearance of larger tumors with combined cisplatin and radiation therapy, the best survival and complete response of HPV-positive tumors was achieved with the addition of Ad-E6/E7. Recently, results of a vaccination trial with viral oncogenes also indicated that vaccination enhances clearance of HPV-transformed cells during chemoradiation therapy. Thus, enhancing the immune response with an anti-HPV oncogene vaccine will likely augment the immunologic clearance induced by combined cisplatin and radiation therapy. Because it may be difficult to obtain approval to inoculate with wild-type oncogenes expressed in adenovirus in humans, an alternative delivery of E6 and E7 antigens could be devised to enhance tumor immune clearance. Such a future approach will likely require a better understanding of how tolerance is initially induced to allow the expression of viral oncoproteins and how to break this tolerance to initiate immune-mediated clearance of the cells containing HPV.

**CONCLUSIONS**

Mouse and human tonsil cells transformed with HPV-16 E6 and E7 oncogenes are less sensitive to radiation and cisplatin in vitro than are HPV-negative tonsil cells. The HPV-positive tumors in mice were more sensitive to combined cisplatin and radiation therapy than were their HPV-negative counterparts and required an intact immune response for tumor clearance. Adoptive transfer of wild-type immune cells into the immune-competent mice restored HPV-positive tumor clearance with cisplatin. Large HPV-positive tumors were cleared in approximately half of the mice treated with concurrent cisplatin and radiation, but the large tumors required the addition of Ad-E6/E7 to achieve clearance in 90% of mice.

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**REFERENCES**


