Protection against HPV-16–Associated Tumors Requires the Activation of CD8⁺ Effector Memory T Cells and the Control of Myeloid-Derived Suppressor Cells

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Abstract

Active anticancer immunotherapeutic approaches have been shown to induce cellular or humoral immune responses in patients, but, thus far, the observed outcomes did not ensure their recommendation for clinical use. The induction of tumor-specific CD8⁺ T cells, although required for the clearance of most solid tumors, was shown to be insufficient for the development of a successful immunotherapeutic approach. The suppressive immune environment triggered by tumors, including the expansion of myeloid-derived suppressor cells (MDSC), is detrimental to the development of antitumor immune responses and precludes the generation of more promising clinical outcomes. In this work, we characterized the CD8⁺ T-cell population specifically involved in the control of tumor growth and the role of MDSCs after administration of an antitumor therapeutic DNA vaccine targeting human papillomavirus type 16 (HPV-16)-associated tumors. Activation of cytotoxic high-avidity CD8⁺ T cells with an effector memory phenotype was found in mice grafted with tumor cells expressing the HPV-16 oncoproteins. In addition, MDSC antibody depletion further enhanced the immunotherapeutic effects of the vaccine, resulting in the complete eradication of tumor cells. Collectively, the current results indicate that the simultaneous control of MDSCs and activation of high-avidity tumor-specific effector memory CD8⁺ T cells are key features for tumor protection by immunotherapeutic approaches and deserve further testing under clinical conditions.

Introduction

Although surgery, chemotherapy, and radiotherapy clearly contribute to cancer control, these therapeutic alternatives are less effective in the treatment of advanced stage tumors. For different types of cancers, an immune response based on the activation of CD8⁺ T cells is generally associated with a positive prognosis of tumor regression (1). Therefore, the development of immunotherapeutic strategies aiming the activation of cytotoxic CD8⁺ T cells represents a major goal in cancer therapy. In this context, active immunotherapeutic approaches represented by anticancer vaccines are target specific and promote milder side effects and long-lasting immune responses. Nonetheless, anticancer vaccines tested in clinical settings have shown moderate activation of cellular immune responses and, more relevantly, reduced clinical outcomes (2). The low efficacy of human anti-cancer vaccine formulations does not seem to be attributed only to the poor induction of antitumor T cells but also to the immunosuppressive tumor microenvironment (3–5).

Tumors inhibit immune responses through different pathways, such as the production of suppressive cytokines and other immune inhibitors that result in the suppression of T-cell responses and/or in the lack of tumor infiltration activity, such as the expansion of myeloid-derived suppressor cells (MDSC; refs. 6, 7). MDSCs are found in increased levels in the blood of patients diagnosed with different cancer types, including head and neck, breast, non–small cell lung, renal, pancreatic, esophageal, gastric cancers, and melanoma (8–12). MDSCs, a heterogeneous population of immature myeloid cells, are capable of interfering with tumor immunity and promoting tumor growth by inhibiting natural killer (NK) cell cytotoxicity and CD4⁺ and CD8⁺ T-cell activation, as well as the activation and attraction of regulatory T cells (Treg), but their actual contribution in the performance of anticancer vaccines is largely unknown (13–16).

Human papillomavirus (HPV) is responsible for approximately 5% of all cancers worldwide and is associated with a subset of anogenital and head and neck cancers (17). Particularly, persistent infections are responsible for all cases of cervical cancer, which is the third leading cause of cancer-related deaths among women worldwide and which accounts for 490,300 diagnoses and 274,000 deaths annually (18, 19). Two preventive anti-viruses HPV vaccines, Gardasil and Cervarix, are commercially available and are expected to have an important impact on cervical cancer prevention in the long term. However, the high cost of these vaccines and the necessity of large-scale immunization programs are expected hurdles to be faced by developing countries, where cervical cancer imposes a greater number of deaths (20). In contrast, antitumor vaccines targeting tumors induced by HPV are currently being tested in different countries; however, thus far, none of the previously tested vaccines has progressed to licensing.
Considering HPV-induced tumors, the E6 and E7 oncoproteins are constitutively expressed in all cancer cells and are required for maintaining the malignant cellular state, thus representing clear targets for immunotherapy. We previously demonstrated that a DNA vaccine encoding the HPV-16 E7 protein genetically fused to the herpes simplex virus 1 (HSV-1) glycoprotein D (gD) protein, coadministered with a vector encoding IL2, induced strong activation of E7-specific CD8+ T cells and promoted full therapeutic protection in mice grafted with tumor cells expressing HPV-16 E6 and E7 (TC-1 cells; ref. 21). In this work, we extended these studies to better define the characteristics of the CD8+ T-cell population associated with the therapeutic eradication of HPV-related tumors. In addition, we assessed the involvement of MDSCs in TC-1 tumor progression and tested whether the depletion of this population could improve the antitumor effects induced by our therapeutic vaccination.

Materials and Methods

Mice

Female C57BL/6 mice at 6 to 8 weeks of age were supplied by the Animal Breeding Center of the Biomedical Sciences Institute of the University of São Paulo (São Paulo, Brazil) and housed at the Parasitology Department of the University of São Paulo. All the procedures involving animal handling followed the recommendations of the University of São Paulo Animal Ethics Committee (protocol number 136-2012).

TC-1 cells

The TC-1 cell line was kindly provided by Dr. T.C. Wu (John Hopkins University, Baltimore, MD) and was transformed with retroviral vectors encoding v-HA-ras and HPV-16 E6 and E7 (22). The TC-1 cells were cultured in DMEM supplemented with 2 mM/L l-glutamine, 1 mM/L sodium pyruvate, 2 mM/L nonessential amino acids, 10 mM/L HEPES buffer, 50 U/mL penicillin/streptomycin, and 10% FBS and were kept at 37°C in an atmosphere of 5% CO2. Before inoculation, the TC-1 cells were harvested by trypsinization, washed twice, and suspended in serum-free media at the proper cell concentration for inoculation. Tumor growth was measured two-dimensionally with a caliper twice a week for at least 60 days. The animals were scored as tumor bearing when the tumors reached a size of approximately 3 mm in diameter. The mice were euthanized once the tumors exceeded a diameter of 15 mm or became necrotic. Vaccination was performed 3 days after the tumor cell injection. Some groups received a booster vaccine dose 40 days after the priming vaccine dose was administered and a rechallenge with 7.5 × 103 TC-1 cells per mice 90 days after the first challenge. The DNA plasmids were delivered intramuscularly, and each dose contained 25 or 50 μg of the DNA vaccine alone or admixed with 25 or 50 μg of the plasmid encoding IL2. The DNA was resuspended in PBS and divided into two 50-μL aliquots that were injected into the tibialis anterior muscle of each mouse hind limb. The depletion of circulating MDSCs was performed on the basis of previously published articles (24). The anti-Gr-1 antibody (clone RB6-8C5) was purified from ascites fluid and delivered systemically by intraperitoneal injections containing 200 μg of antibody once a week for six injections total starting 3 days after tumor cell engrafment. Nonvaccinated and immunized mice received intraperitoneal injections of purified rat IgG as control. Alternatively, vaccination in combination with administration of anti-Gr-1 antibody initiated 7 or 10 days after the tumor cell injection. In this case, two doses of 50 μg of plasmids pgDE7 and pIL2 and 5 doses of anti-Gr-1 antibody, both at weekly intervals, were applied to the animals.

Flow cytometry

Blood and spleen samples were collected at different time points after the vaccine administration and treated for red blood cell lysis. Cells were surface stained with anti-CD11b and anti-Gr-1 (RB6) for the detection of the MDSC population and cell sorting (FACSaria). MDSC subsets were determined using anti-CD11b, anti-Ly6C (AL21), and Ly6G (1A8). Intracellular IFNγ staining was performed using blood and spleen samples cultured over-night at 37°C with 10 μg/mL brefeldin A (GolgiPlug; BD Biosciences) with or without 1.5 μg/mL E7-specific RAHYNIVTF peptide (amino acids 49–57). Alternatively, serial dilutions of the peptide were used as stimuli for the assessment of CD8+ T-cell avidity. In some experiments, fluorescently labeled anti-CD107a and 10 μg/mL monensin (GolgiStop; BD Biosciences) were also added during the in vitro stimulation. After in vitro stimulation, surface staining was performed using fluorescently labeled antibodies specific for mouse CD8, CD62L, and CD44. After permeabilization, the cells were incubated with anti-IFNγ conjugated to a different fluorochrome. To evaluate the inhibition of IFNγ production by CD8+ T cells in the presence of MDSCs, 5 × 105 sorted CD11b+Gr-1+1TC-1 cells from spleen samples of tumor-bearing mice were plated with the same number of splenocytes from mice immunized with pgDE7+pIL2 (responder cells) and the E7-derived peptide in the conditions described above. Tumor samples were collected 14 days after tumor cell injection in Matrigel (BD Biosciences), digested with collagenase A (1 mg/mL), and macerated to obtain a cell suspension. Samples were stained with E7-specific APC-conjugated MHC class I Dextramer (Immudex) and surface stained with anti-CD8. The buffers and antibodies were purchased from BD Biosciences. The samples were examined by flow cytometry using a FACSCalibur or FACSCan (BD Biosciences), and data were analyzed using FlowJo software (TreeStar).

DNA vaccines

The DNA vaccine encoding the HPV-16 E7 protein genetically fused after amino acid 244 of the HSV-1 gD protein has been described previously (21, 23). The gene sequence encoding the gDE7 chimeric protein was cloned into the pVAX1 vector (Invitrogen), which contains a cytomegalovirus (CMV) promoter and a kanamycin resistance gene. The plasmid encoding murine IL2, which was kindly provided by Dr. Sergio Costa Oliveira (University of São Paulo, Ribeirão Preto, Brazil), encodes murine IL2 in a pcDNA3 plasmid background (Invitrogen) that contains the CMV promoter and an ampicillin resistance gene.

Immunization regimen and tumor cell challenge

Groups of 5 to 10 mice were challenged subcutaneously with 7.5 × 103 TC-1 cells per mice resuspended in 100 μL of serum-free media and injected into the right rear flank of the mouse. For the tumor infiltration experiments, the same amount of TC-1 cells was diluted in 200 μL of serum-free media and 200 μL of Matrigel.
Figure 1.
Administration of pIL2 and pgDE7 induces higher activation of E7-specific Tem CD8⁺ T cells. Groups of 5 mice were challenged with 7.5 x 10⁴ TC-1 cells and immunized 3 days later with one dose of pgDE7, pIL2, or both plasmids combined (50 μg of each plasmid). Spleens were harvested 14 days after vaccination, or blood was collected weekly after challenge. Splenocytes and blood cells were stimulated in vitro overnight with a peptide from HPV-16 E7 corresponding to the K⁰ MHC class I-restricted epitope. A, percentage of CD8⁺ T cells producing IFNγ or simultaneously IFNγ and CD107a from splenocytes. B, percentage of CD8⁺ T cells producing IFNγ after stimulation with serial dilutions of E7 peptide from splenocytes. C, percentage of CD8⁺ T cells with a Tcm or Tem phenotype from splenocytes. Numbers on top, Tem/Tcm ratio. D, percentages of IFNγ-producing Tcm or Tem cells; Tcm, central memory CD8⁺ T cells (CD8⁺CD44highCD62L⁺); Tem, effector memory CD8⁺ T cells (CD8⁺CD44hiCD62L⁻). E, percentage of IFNγ-producing CD8⁺ T cells over time from blood samples. The data are from one representative experiment of three performed. The data are expressed as the mean ± SD. *, P < 0.05; **, P < 0.005; ***, P < 0.001; ns, not significant.
Statistical analyses

ANOVA was employed, followed by Tukey posttest, when individual data sets were compared. Log-rank tests were performed whenever survival curves were compared. \( P < 0.05 \) was considered significant.

Results

Antitumor protective responses are linked to tumor-specific, high-avidity, and IFN\( \gamma \)-producing effector memory CD8\( ^+ \) T cells

We previously showed that the coadministration of a plasmid encoding IL2 with a DNA vaccine encoding the HPV-16 E7 protein genetically fused to HSV-1 gD (pgDE7) generated full therapeutic antitumor protection after a single vaccine dose (21). In contrast, under the same conditions, immunization with the vaccine vector without coadministration of the plasmid encoding IL2 resulted in the activation of similar numbers of E7-specific CD8\( ^+ \) T cells but did not confer therapeutic antitumor protection. To further investigate the characteristics of the effective antitumor immune response elicited in the mice immunized with the two plasmids, we immunized TC-1 cell–grafted mice with one dose of pgDE7 combined with plL2 or each plasmid separately 3 days after tumor cell engraftment. Two weeks after the immunization, splenocytes were cultured in the presence of the E7-specific peptide corresponding to the immunodominant MHC class I–restricted epitope, and the CD8\( ^+ \) T cells induced in mice of different immunization groups were characterized. CD8\( ^+ \) T cells of mice immunized with pgDE7 coadministered with plL2 showed significantly higher IFN\( \gamma \) and CD107a production than cells from animals of the other immunization groups, thus indicating stronger activation and degranulation of these cells (Fig. 1A). Similarly, mice immunized with the two vectors generated CD8\( ^+ \) T cells with stronger avidity to the target peptide because IFN\( \gamma \)-producing CD8\( ^+ \) T cells were detected using a 10-fold lower concentration of the E7-derived peptide (0.15 \( \mu \)g/mL) in contrast with cells collected from animals immunized with pgDE7 alone (15 \( \mu \)g/mL; Fig. 1B). In addition, the coadministration of plL2 in combination with pgDE7 shifted the phenotype of the E7–specific CD8\( ^+ \) T cells to a more pronounced T effector memory (Tem) phenotype (CD8\( ^+ \)CD44\( ^{high} \)CD62L\( ^{low} \)) compared with a central memory (Tcm) CD8\( ^+ \) T-cell phenotype (CD8\( ^+ \)CD44\( ^{high} \)CD62L\( ^{hi} \); Fig. 1C). Importantly, the Tem CD8\( ^+ \) T-cell population showed stronger IFN\( \gamma \) production (Fig. 1D).

Mice immunized with pgDE7 combined with plL2 showed a higher IFN\( \gamma \) response peak around 14 days after immunization in comparison with mice immunized with pgDE7 alone (Fig. 1E). The longevity of IFN\( \gamma \) production by CD8\( ^+ \) T cells was not altered by the coadministration of plL2, regarding mice immunized only with pgDE7 (Fig. 1E). As expected, tumor protection correlated with the number of CD8\( ^+ \) T cells infiltrating into tumors, which was significantly enhanced in mice immunized with pgDE7 in combination with plL2 (Fig. 2A and B). Importantly, in these mice, more than 30% of the tumor-infiltrating CD8\( ^+ \) T cells showed to be E7 specific, as demonstrated by the binding to the E7 peptide MHC class I dextramer (Fig. 2C and D).

Protection against tumor rechallenge requires a booster dose and the reinforcement of the effector memory CD8\( ^+ \) T-cell response

Ideally, an antitumor vaccine should be capable of controlling preexistent lesions and avoid tumor recurrences. To determine whether the combination of pgDE7 and plL2 could promote protection against tumor relapses, we grafted an additional load of TC-1 cells 90 days after the first challenge in mice immunized
with the two DNA vectors. In the group administered with one dose of the vaccine, 70% of the animals remained tumor free; however, full tumor recurrence protection was achieved only in mice that received a second vaccine dose 40 days after the priming dose (Fig. 3A). The monitoring of IFN-\(\gamma\) production by CD8\(^{+}\) T cells showed that the booster dose induced a second peak of E7-specific IFN-\(\gamma\)-producing CD8\(^{+}\) T cells, which were maintained during the following 2 weeks and displayed a less sharp contraction phase than that observed after the priming dose (Fig. 3B). Moreover, mice immunized with one or two doses of the vaccine formulation showed a CD8\(^{+}\) T-cell activation response peak one week after the rechallenge with TC-1 cells. Nonetheless, mice that received the booster dose showed higher numbers of IFN-\(\gamma\)-producing CD8\(^{+}\) T cells after the second tumor cell transplantation (Fig. 3B). The administration of the second vaccine dose did not change the longevity of the CD8\(^{+}\) T-cell response and E7-specific IFN-\(\gamma\) production (Fig. 3B). Supporting the observation made with mice immunized with a single vaccine dose, the protective antitumor immunity of mice submitted to the prime-boost immunization regimen correlated with more efficient Tem CD8\(^{+}\) T-cell activation and a 3-fold increase in IFN-\(\gamma\) production by these cells compared with the Tcm CD8\(^{+}\) T-cell population (Fig. 3C and D). The results indicate, thus far, that the activation of IFN-\(\gamma\)-producing CD8\(^{+}\) T cells with increased cytotoxic effects is essential for the promotion of tumor protection. Moreover, increased frequencies of CD8\(^{+}\) T cells displaying an effector memory phenotype correlated with more efficient control of primary tumors and recurrences.

Tumor control promoted by vaccination with pgDE7 and pIL2 avoids accumulation of MDSCs

Tumor progression is often associated with the expansion of immunosuppressive cell populations that can negatively impact the responses induced by anticancer vaccines. Under our experimental conditions, tumor-bearing mice showed a large expansion of CD11b\(^{+}\)Gr1\(^{+}\) MDSCs 2 months after tumor cell transplantation. In mice inoculated with PBS or pIL2, the MDSC
Figure 4.
Administration of pgDE7 and plL2 avoids MDSC expansion. Groups of 5 mice were challenged with 7.5 × 10^4 TC-1 cells and immunized 3 days later with one dose of pgDE7, plL2, or both plasmids combined (50 µg of each plasmid). Splenocytes and blood cells were surface stained with anti-CD11b and anti-Gr-1 conjugated to different fluorochromes. 

A, dot plot representing the percentage of CD11b^+ Gr-1^+ cells in the spleen of one representative mouse at 60 days after challenge. 

B, percentage of CD11b^+ Gr-1^+ cells was determined from splenocytes collected at 14 or 60 days after challenge; these percentages are expressed as the mean ± SD. 

C, images of tumors and spleens of two representative mice of each immunization group 60 days after challenge. 

D, percentages of CD11b^+ Gr-1^+ cells over time in blood samples collected weekly after challenge with TC-1 cells. **, comparison of plL2 versus PBS and plL2 groups. 

E, tumor sizes were measured 2 to 3 times a week for 30 days after TC-1 cell challenge. Comparison of pgDE7 or pgDE7 + plL2 versus PBS and plL2 groups. 

F, correlation curve and linear regression for the percentage of CD11b^+ Gr-1^+ cells and tumor size (PBS, r^2 = 0.56; plL2, r^2 = 0.56). 

G, percentages of the granulocytic (CD11b^+ Ly6G^+ Ly6C^low) and the monocytic (CD11b^+ Ly6G^+ Ly6C^hi) subsets of MDSCs from spleen samples 30 days after tumor cells graft. The data are from one representative experiment of at least two performed. *, P < 0.05; **, P < 0.005; *** P < 0.001; ns, not significant.
Figure 5.
MDSC depletion enhances the antitumor protective effects of the pgDE7 vaccine. Groups of 5 mice were challenged with $7.5 \times 10^4$ TC-1 cells and immunized 3 days later with one dose of pgDE7 or pgDE7 admixed with pIL2 combined with or without MDSC depletion. **A**, anti-Gr-1 mAb or the isotype control (Iso) was administered intraperitoneally 3 days after challenge, followed by 4 weekly injections. **A** and **B**, control mice (PBS) or mice immunized with one dose of 50 mg of pgDE7 were treated with anti-Gr-1 mAb or the isotype control. **A**, tumor sizes were measured 2 to 3 times a week for 30 days after TC-1 cell challenge. ’ comparison of PBS versus pgDE7 + αGr-1 group. **B**, percentage of CD11b+Gr-1+ cells from spleens harvested 30 days after tumor challenge. **C** and **D**, sham-treated mice (PBS) or mice immunized with one dose of 25 μg of pgDE7 were treated with or without anti-Gr-1 mAb and/or 25 μg of pIL2. **C**, percentage of tumor-free mice in the therapeutic antitumor protection model. Tumor development was followed for 60 days after challenge. Comparison of each group versus pgDE7 + pIL2 + αGr-1 group. (Continued on the following page.)
The data are from one representative experiment of at least two performed. The data are expressed as the mean percentage of IFNγ+ CD8+ T cells from spleens harvested 14 days after the immunization. E, mice were immunized with two doses of 50 μg of pgDE7 and pIL2 combined or not with anti-Gr-1 mAb, starting 7 or 10 days after tumor cell injection. Data are expressed as percentages of tumor-free mice.

Comparison of each group versus pgDE7 + pIL2 seven days group. F, percentage of IFNγ+ CD8+ T cells from spleens harvested 14 days after the immunization with pgDE7 + pIL2. Cells were not stimulated (−) or incubated in vitro with the E7-derived peptide alone (E7) or in the presence of MDSCs (E7 + MDSC). The data are from one representative experiment of at least two performed. The data are expressed as the mean ± SD. *P < 0.05; **P < 0.005; ***P < 0.0001, ns, not significant.

Depletion of MDSCs delays tumor growth and enhances the efficacy of the DNA vaccine pgDE7. Current immunotherapeutic approaches targeting immunosuppressive mechanisms show promising results in the control of different cancer types (25). To evaluate the potential antitumor effects of MDSC depletion, the anti-Gr-1 mAb was administered to mice grafted with TC-1 cells. The treatment with anti-Gr-1 mAb alone significantly reduced the percentages of MDSC populations in the spleen and promoted tumor growth delay but could not completely prevent tumor development (Fig. 5A and 5B).

To determine whether depletion of MDSCs could function as a neoadjuvant therapy in conjunction with the anticancer vaccine, mice transplanted with TC-1 cells were immunized with a single dose of pgDE7 at a suboptimal quantity (25 μg of each plasmid) with or without the coadministration of pIL2 and MDSC depletion. Mice immunized with pgDE7 did not show any antitumor protection effect; however, the depletion of MDSCs reduced mortality to 50% (Fig. 5C). Similarly, the coadministration of pgDE7 and pIL2 at suboptimal quantities resulted in 60% anti-tumor protection (Fig. 5C). Nonetheless, mice therapeutically immunized with pgDE7 and pIL2 at suboptimal quantities showed full eradication of previously established tumors (Fig. 5C).

In addition, the association of the anti-Gr-1 mAb treatment with the active immunotherapy resulted in stronger activation of the E7-specific CD8+ T-cell responses in mice immunized with pgDE7 alone or co-administered with pIL2 (Fig. 5D). Importantly, depletion of MDSCs in addition to the treatment with pgDE7 and pIL2 increased the protection to established tumors. Groups of mice immunized with the combined therapy 7 or 10 days after tumor cell injection, displayed 50% and 30% tumor protection, respectively (Fig. 5E).

(Continued)
or admixed with pgDE7 did not increase the numbers of CD4+ FoxP3+ cells in tumor-bearing mice and did not increase the IL2 serum levels (data not shown). Although IL2 plays a dual role by eliciting pro- and anticancer immune responses, accumulating evidences indicate that this cytokine favors the enhancement of cytolytic CD8+ T-cell activation under in vivo conditions (30, 31).

In this work, the coadministration of pIL2 and pgDE7 enhanced the expression of IFNγ and CD107a by E7-specific CD8+ T cells, which correlated with stronger activation and degranulation activities in these cells. These evidences support the anticancer effect of IL2 in combination with active immunotherapeutic approaches.

Although protective antitumor responses in both mice and humans are often associated with CTL induction, the generation of high-frequency T-cell responses is not necessarily an indication of a protective response. Some groups have shown that T-cell functional avidity can better correlate with an efficient antitumor immune response (32–34). In agreement with this observation, our data showed that a more efficient antitumoral response correlated with the activation of CD8+ T cells with higher antigen avidity. A study comparing peptide, peptide-pulsed DCs, and DNA vaccines showed that the immunization of mice with a plasmid-based strategy resulted in higher avidity T-cell responses to specific epitopes (35). This feature was linked to the capacity of DNA vaccines to promote both direct and cross-presentation of epitopes by transfected antigen-presenting cells (36). In our work, the strongest antitumor protective effects correlated with the higher antigen avidity of CTLs as well as with the CD8+ T-cell tumor infiltration activity, particularly E7-specific CD8+ T cells, in accordance with previous observations (37, 38).

In our study, the administration of pgDE7 vaccine induced a more pronounced Tem phenotype (CD8+ CD44highCD62L−) and a higher activation of these cells compared with Tcm CD8+ T cells (CD8+ CD44highCD62L+). The phenotype polarization of T cells is influenced by the cytokine milieu and secondary signals promoted by coinhibitory and costimulatory receptors, including the herpesvirus entry mediator (HVEM; refs. 39, 40). The HSV-1 gD protein, expressed by the vaccine formulation studied here, was shown to exert CD8+ T-cell activation through binding to HVEM (41). The gD/HVEM interaction induces NF-kB activation and blockade of CD160 and BTLA (B and T lymphocyte attenuator) coinhibitory signals, contributing to the induction of T-cell effector responses (39, 40, 42). Furthermore, our results demonstrated that IL2 coexpression combined with the pgDE7 DNA vaccine accentuated the CD8+ T-cell effector phenotype. The role of IL2 in modulating CD8+ memory T cells had only been shown in vitro and in combination with IL15 (43). In addition, the CD8+ Tem phenotype was associated with a higher therapeutic antitumor effect and with tumor recurrence control. The induction of CD8+ effector memory cells could confer an advantage for anticancer therapies because this population has immediate effector function compared with central memory cells (44, 45). Similarly, a work conducted with an HPV-related peptide-based anticancer vaccine showed that formulations containing the Toll-like receptor (TLR) ligands CpG (TLR9) and poly-IC (TLR3) induced increased effects and higher frequencies of effector memory cells (CD62L− KLRG1+) capable of secreting IFNγ and TNF (46).

In the mouse tumor model used in this study, tumor progression induced the accumulation of MDSCs, which have been shown to reduce CD8+ T-cell activity and, thus, can be detrimental to eradication of tumors at more advanced stages. In our study, CD8+ T cells were less responsive to antigenic stimulation in the presence of MDSCs. In the TC-1 tumor model, the expansion of MDSCs was shown to inhibit CD8+ T-cell activation through NODependent mechanisms (47). The increase of the MDSC population and the circulation of these cells in the blood showed a positive correlation with tumor size. In contrast, the control of tumor growth induced by the administration of the immunotherapeutic treatment avoided the expansion of this cell population in either the spleen or blood. In the tumor model used in this study, both the monocytic and granulocytic subpopulations of MDSCs expanded following tumor growth. A larger accumulation of the granulocytic phenotype was observed in tumor-bearing mice, which was observed before for different tumor models (48). An increase in the number of circulating MDSCs has been reported in different cancers types, particularly among patients with advanced disease stages, and inversely correlated with survival (49).

The mechanisms of evasion or suppression triggered by tumors, including those mediated by MDSC, can help to explain why cancer vaccines have been relatively unsuccessful for the treatment of patients with established disease. Therefore, immunotherapeutic strategies designed to control tumors should not only induce an efficient antitumor immune response but also avoid immunosuppressive cell population proliferation. The depletion of MDSCs promoted tumor growth delay even in mice nonimmunized with the DNA vaccine, further supporting a direct role for these cells in tumor cell growth in this experimental model. MDSC depletion through the administration of an anti-Gr-1 mAb showed a synergistic antitumor effect with the pgDE7 vaccine formulation, inducing higher IFNγ production by CD8+ T cells and full therapeutic protection using lower vaccine doses or treating mice with more advanced tumors. Similar results were found using a model based on OVA-expressing lung carcinoma cells, where MDSC depletion combined with an anti-OVA vaccine increased antitumor protection and recurrences due to enhanced IFNγ secretion by CD8+ T cells (24). However, we did not find another work that combined anti-Gr1 treatment with an active immunotherapy targeting actual tumor antigens. In addition, targeting different factors related to the expansion of the MDSC population, including stem cell factor, COX-2, and prostaglandins, as well as macrophage CSF, IL1β, IL6, GM-CSF, and VEGF (50), may be additional alternatives for enhancing the protective effects conferred by therapeutic anticancer vaccines.

Our work demonstrated that the induction of CD8+ T cells with high avidity and with a Tem phenotype by a therapeutic anticancer vaccine correlates with an enhanced antitumor effect. Furthermore, the depletion of MDSCs can play an adjuvant role for active immunotherapy, leading to the more efficient elimination of rapidly growing tumor cells, such as those frequently found at advanced stage HPV-related cancers. Collectively, these results provide relevant experimental evidence that can contribute to the development of an alternative immunologic treatment with greater chances of clinical success in trials aimed to control HPV-induced tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: M.O. Diniz, L.C.S. Ferreira

Development of methodology: M.O. Diniz
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.O. Diniz, N.S. Sales, J.R. Silva

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.O. Diniz, N.S. Sales

Writing, review, and/or revision of the manuscript: M.O. Diniz, N.S. Sales, L.C.S. Ferreira

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.O. Diniz

Study supervision: L.C.S. Ferreira

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