International Immunopharmacology 69 (2019) 279–288

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Prevention and treatment of cervical cancer by a single administration of human papillomavirus peptide vaccine with CpG oligodeoxynucleotides as an adjuvant *in vivo*

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ARTICLE INFO

Keywords:
Adjuvant
Cervical cancer
CpG oligodeoxynucleotides
Human papillomavirus
Peptide
Vaccine

ABSTRACT

No licensed therapeutic human papillomavirus (HPV) vaccine is currently available, so it remains a high priority to develop a therapeutic HPV vaccine or prophylactic/therapeutic HPV vaccine for cervical cancer. In this current study, we designed an HPV vaccine including CpG oligodeoxynucleotides 1826 as an adjuvant and HPV16 E7 43-77 peptide as antigen, which contains a CD8 T cell epitope (E7 49-57), and two CD4 T cell epitopes (E7 43-77 and E7 50-62). The prophylactic and therapeutic effect on cervical cancer induced by a single administration of vaccine, were comprehensively evaluated by examining the tumor size and the percentage of tumor-free/bearing mice. The cellular immunity and modulation of immunosuppressive cells induced by the vaccine were evaluated by examining intracellular cytokine staining (ICS) of splenocytes and FCM, respectively. Antigen-specific cytotoxic T-lymphocyte (CTL) responses were investigated using *in vivo* cytolytic assay. The results showed that the single administration of vaccine elicited significant prophylactic as well as therapeutic effect on cervical cancer. The increased cellular immunity mediated by CD4 + IFN-γ + T cells and CD8 + IFN-γ + T cells, and the decreased numbers of immunosuppressive cells including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) were induced by the vaccine. Antigen-specific CTL response was also induced by vaccination. These findings suggested that significant anti-tumor effect of the vaccine may result from the induction of increased cellular immunity and decreased immunosuppressive cells.

1. Introduction

Cervical cancer, the fourth most common cancer in women worldwide [1], is associated with a persistent infection of “high-risk” human papillomavirus (HPV) [2]. Thus far, several HPV prophylactic vaccines have been approved for the prevention of HPV infection. But due to a variety of factors including negative patients' attitudes, beliefs and high cost [3], acceptance and utilization of the vaccine in a population remain low. Moreover, the prophylactic vaccines have no therapeutic effect on the established HPV infections or HPV-associated tumors [4–6]. Based on the fact that no licensed therapeutic HPV vaccine is currently available, it remains a high priority to develop a therapeutic HPV vaccine or prophylactic/therapeutic HPV vaccine for cervical cancer.

Of all the “high-risk” HPVs identified, HPV type 16 (HPV16) is one of the most common type and its persistent infection is responsible for virtually all cervical cancers worldwide [7]. The high risk HPV E7 oncoprotein degrades tumor suppressor pRB in cells and its expression is essential for cellular malignant transformation [8]. HPV E7 protein is highly conserved [9] and constitutively expressed on HPV-infected cells or HPV-transformed tumor cells, but not on normal cells. Therefore, it represents an ideal target of immunotherapy for cervical cancer and its precursor lesions.

CpG oligodeoxynucleotides (ODN) directly and indirectly activate TLR9-expressing B cells and plasmacytoid dendritic cells (pDC) [10], a special type of dendritic cell, leading to the production of the type I interferon (IFN) that is important for clearing intracellular pathogens [11]. CpG ODN-activated B cells and pDC show upregulation of co-stimulatory molecules, the chemokine receptor CCR7, and increase secretion of Th1-like proinflammatory cytokines, IFNγ-inducible 10-kDa protein [12], which transform T cells from tolerance to a strong cytotoxic T-lymphocyte (CTL) response against the tumor antigens.

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https://doi.org/10.1016/j.intimp.2019.01.024
Received 7 November 2018; Received in revised form 10 January 2019; Accepted 15 January 2019
Available online 08 February 2019
1567-5769/

Therefore, CpG ODN as potent Th1 immune response immunostimulator was incorporated in many tumor therapeutic vaccines [13].

In this current study, we designed an HPV vaccine including CpG ODN 1826 as an adjuvant and HPV 16 E7 43-77 peptide as antigen, which contains a CD8 T cell epitope (E7 49-57) [14,15], and two CD4 T cell epitopes (E7 43-77 [16] and E7 50-62 [16]).The prophylactic and therapeutic effect on cervical cancer as well as immune responses induced by a single administration of vaccine, were comprehensively evaluated. The results showed that the single administration of vaccine elicited prophylactic as well as therapeutic effect on cervical cancer. The increased cellular immunity and the decreased numbers of immunosuppressive cells including macrophages, myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) induced by the vaccine were demonstrated.

2. Materials and methods

2.1. Mice and cell lines

Female C57BL/6 mice aged 6–8 weeks were purchased from Liaoning Changsheng Biotechnology Co. Ltd. (Benxi, China). All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper use and care of laboratory animals. The TC-1 cell line was purchased from Beijing Beina Chuanglian Biotechnology Institute (Beijing, China). Cells were grown in culture with Roswell Park Memorial Institute (RPMI) 1640, 10% fetal calf serum, 2 mM G418, 100 U/ml penicillin and 100 g/ml streptomycin at 37 °C and 10% carbon dioxide.

2.2. Peptides and adjuvant

The HPV16 E7 peptides (E7 49-57 and E7 43-77) and OVA peptide were synthesized by the Fmoc solid-phase method at GL Biochem (Shanghai) Ltd. (Shanghai, China). Peptide purity was determined to be > 95% by high-pressure liquid chromatography (HPLC) and peptide sequences were validated by mass spectrometry (MALDI-TOF). Lyophilized peptides were dissolved in a small amount of DMSO followed by addition of water. CpG-ODN 1826 was synthesized with a nuclease-resistant phosphorothioate backbone by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). The sequence of peptides and CpG ODN as follows:

E7 49-57: RAHYNIVTF;
E7 43-77: GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR;
OVA 257-264: SIINFEKL;
CpG-ODN1826: 5′-TCCATGACGTTCCTGACGTT-3′.

2.3. In vivo tumor growth experiments

2.3.1. Therapeutic experiment

The C57BL/6 mice were randomly divided into 8 groups. On day 0, all mice were injected subcutaneously with 5×10^5 TC-1 cells in the right flank. On day 4, the groups of mice were injected subcutaneously with vaccinations in the contralateral flank. Immunization dose contained 20 μg per dose of CpG-ODN alone, 20 μg per dose of CpG-ODN mixed with 10 μg, 30 μg and 50 μg per dose of peptides (short peptide covering minimal CTL epitope E7 49-57 and long peptide E7 43-77). We referred to the vaccine including CpG-ODN and E7 49-57 as short peptide vaccine and the vaccine including CpG-ODN and E7 43-77 as long peptide vaccine. The control group was injected with PBS. Tumors were measured 2 to 3 times per week with digital calipers spanning the shortest and longest surface diameters. Tumor size was calculated from two measurements using the following formula: tumor volume (mm^3) = $0.5 \times a \times b^2$ (a is the longest diameter of the tumor, b is the shortest diameter of the tumor). The percentage of tumor-bearing mice is the number of tumor-bearing mice in each group divided by the total number of mice in the group and multiplied by 100%.

2.3.2. Prophylactic experiment

The mice were divided into 4 groups. On day 0, all mice were immunized subcutaneously. Immunization dose contained 20 μg per dose of CpG-ODN alone, 20 μg per dose of CpG-ODN mixed with 50 μg per E7 49-57 peptide and E7 43-77 peptide. The control group was injected with PBS. One week after the vaccination, mice were injected subcutaneously with 5×10^5 TC-1 cells suspended in 0.1 ml PBS. Tumor growth was monitored 2 to 3 times per week.

2.4. Intracellular cytokine staining

On day 28, the mice were euthanized and spleens were harvested. After lysis of the red blood cells (RBCs), the dissociated cells were dispersed with 1 ml of PBS (1×10^6/ml). For analysis of IFN-γ cytokine, the splenocytes were incubated with the Phorbol 12- myristate 13-acetate (PMA, 1 μg/ml) (Sigma-Aldrich, St. Louis, MO, USA) and Ionomycin (50 μg/ml, Sigma-Aldrich, St. Louis, MO, USA) for 5 h, Golgiplug (BD Biosciences, San Diego, CA, USA) for the final 4 h. After stimulation, the splenocytes were stained with the following fluorescein-conjugated antibodies: PerCP-conjugated anti-mouse CD8a (Biolegend, San Diego, CA, USA), FITC-conjugated anti-mouse CD4 (Biolegend, San Diego, CA, USA), PE-conjugated anti-mouse IFN-γ (Biolegend, San Diego, CA, USA). Analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson, USA) and analyzed by FlowJo X software (TreeStar, Ashland, OR).

2.5. Intranuclear transcription factors staining

Single-cell suspension of splenocytes was prepared as described above. For analysis of Treg cells, surface dye of freshly isolated splenocytes was performed by adding FITC-conjugated anti-mouse CD4 (Biolegend, San Diego, CA, USA), Percp-conjugated anti-mouse CD25 (Biolegend, San Diego, CA, USA), and intranuclear staining was carried out by adding PE-conjugated anti-mouse FoxP3 (Biolegend, San Diego, CA, USA) after fixation and membrane permeation. Analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson, USA) and data were analyzed by FlowJo X software (TreeStar, Ashland, OR).

2.6. Surface molecules staining

Single-cell suspension of splenocytes was prepared as described above. For analysis of surface molecules associated with myeloid-derived suppressor cells (MDSCs) and macrophage, splenocytes were stained with FITC-conjugated anti-mouse Gr-1 (Biolegend, San Diego, CA, USA), Percp-conjugated anti-mouse CD11b (Biolegend, San Diego, CA, USA), PE-conjugated anti-mouse F4/80 (Biolegend, San Diego, CA, USA). Analysis was performed as described above.

2.7. In vivo cytotoxic T lymphocyte (CTL) response

In brief, splenocytes from naive C57BL/6 mice were collected and loaded with either 10 μM E7 43-77 or Ova 257-264 peptide in complete media at 37 °C for 1 h. Then, the E7 43-77 peptide-pulsed and Ova peptide-pulsed cells were labeled with 4 μM and 0.4 μM CFSE, respectively. Equal amount of $CFSE^{high}$ (E7 43-77 pulsed cells) and $CFSE^{low}$ (Ova pulsed cells) were mixed and i.v. injected into the untreated and treated mice. Eighteen hours later, splenocytes were analyzed by flow cytometry and enumerated according to a published equation [17].

$$\%specific\ lysis = (Ova \times x - E7\ 43 - 77)/(Ova \times x) \times 100\%$$

where x = E7 43–77/Ova from native mice.

2.8. Statistical analysis

Calculations were performed using Prism software, version 5.0 (Graph-Pad Software, San Diego, CA). Data were described as means ± standard deviation (s.d.). Statistical significance of percentage of tumor-free/bearing mice was performed using the log-rank test. Statistical significances of tumor size, immune cells and CTL results were determined with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A difference between groups was considered significant at $P < 0.05$.

3. Results

3.1. Tumor regression induced by vaccination

To determine whether vaccination could affect the growth of established tumors, 5×10^5 TC-1 cells were used to establish tumors in the right flank of C57BL/6 mice. On 4th day, the mice were treated with vaccines containing CpG ODN and different doses of long/short peptides or PBS as a control. In groups treated with 10 μg, 30 μg, 50 μg long peptides and 30 μg, 50 μg short peptides vaccine, inhibition of tumor growth was shown significantly compared with control group. CpG-ODN alone and 10 μg short peptide vaccine didn't induce the inhibition of tumor growth (Fig. 1A). The tumor-bearing percentage curve demonstrated that as of the 24th day of the experiment, all mice in the PBS group had tumors and the largest tumor volume sized up to 1697.23 mm^3. Five of the six mice in the 10 μg long peptide, 10 μg short peptide vaccine groups and 30 μg short peptide vaccine group, two of the six mice in the 30 μg long peptide vaccine group and three of the six mice in the 50 μg short peptide vaccine group had tumors. All of mice treated with 50 μg long peptide vaccine eradicated the tumors completely (Fig. 1B). Comparison of tumor size and the percentage of tumor-bearing mice showed that there was no significant difference between mice treated with 50 μg long peptide and 50 μg short peptide vaccine, but vaccine containing 50 μg long peptide induced more efficacious therapeutic effect on cervical cancer considering the complete eradication induced by the long peptide vaccine.

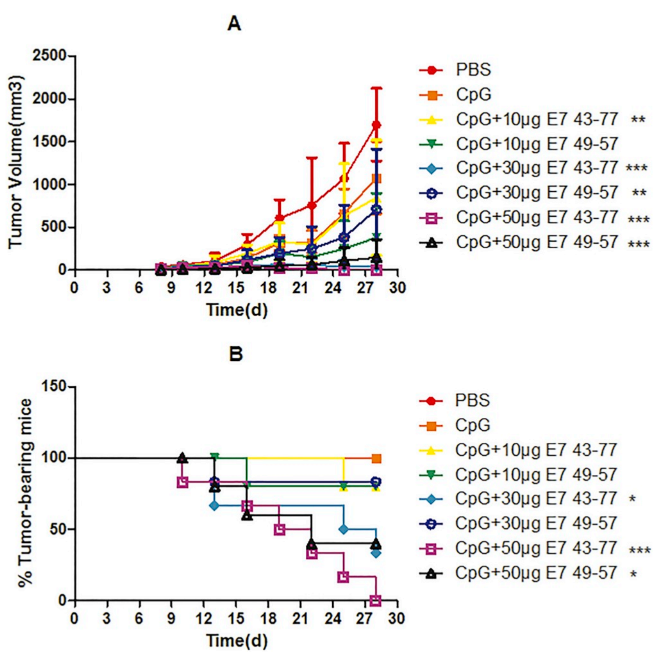


Fig. 1. Inhibition of tumor growth induced by vaccination. On day 0, 5×10^5 TC-1 cells were injected subcutaneously to the right flank of the mice ($n = 6$). On day 4, vaccines were injected subcutaneously into the left flank of the mice. The control group received the same dose of PBS. Vaccine inhibited tumor growth, and the 50 μg long peptide vaccine group showed the best tumor growth inhibition effect considering the complete eradication of tumor. (A), the tumor volume of each group in mice. Data are shown as mean ± s.d. Statistically significant differences (**$P < 0.01$, ***$P < 0.001$) are indicated. (B), the percentage of tumor-bearing mice (*$P < 0.05$, ***$P < 0.001$).

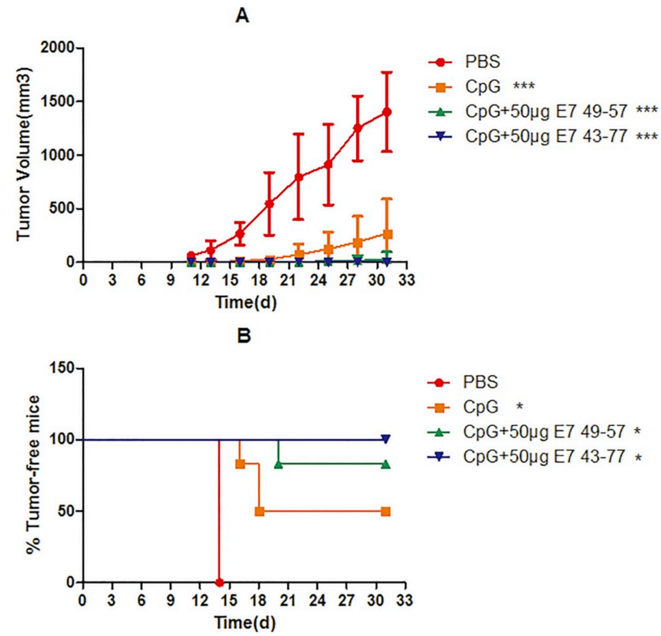


Fig. 2. Prevention of tumor induced by the vaccine. On day 0, the vaccines were injected subcutaneously to the left flank of the vaccinated mice ($n = 6$). The control group did not receive any vaccine injection but the same dose of PBS. On day 7, 5×10^5 TC-1 cells were injected subcutaneously to the right flank of the mice. (A), the tumor volume of each group in mice. Data are shown as mean ± s.d. Statistically significant differences (***$P < 0.001$) are indicated. (B), the percentage of tumor-free mice (*$P < 0.05$).

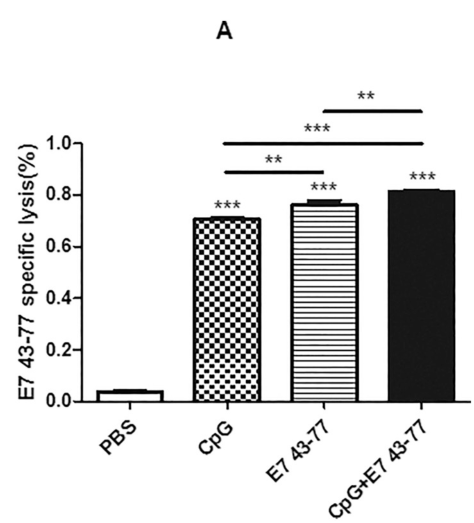


Fig. 3. *In vivo* CTL response induced by the vaccine. Splenocytes from naïve mice were pulsed with Ova or E7 43-77 peptide and stained with low (Ova) or high (E7 43-77) concentrations of CFSE, respectively. The cells were then mixed and injected into the vaccinated mice ($n = 4$). After 18 h, splenocytes from the vaccinated mice were analyzed by flow cytometry and enumerated according to a published equation. Representative graph from each group is shown. Data are shown as mean ± s.d. Statistically significant differences (** $P < 0.01$, *** $P < 0.001$) are indicated.

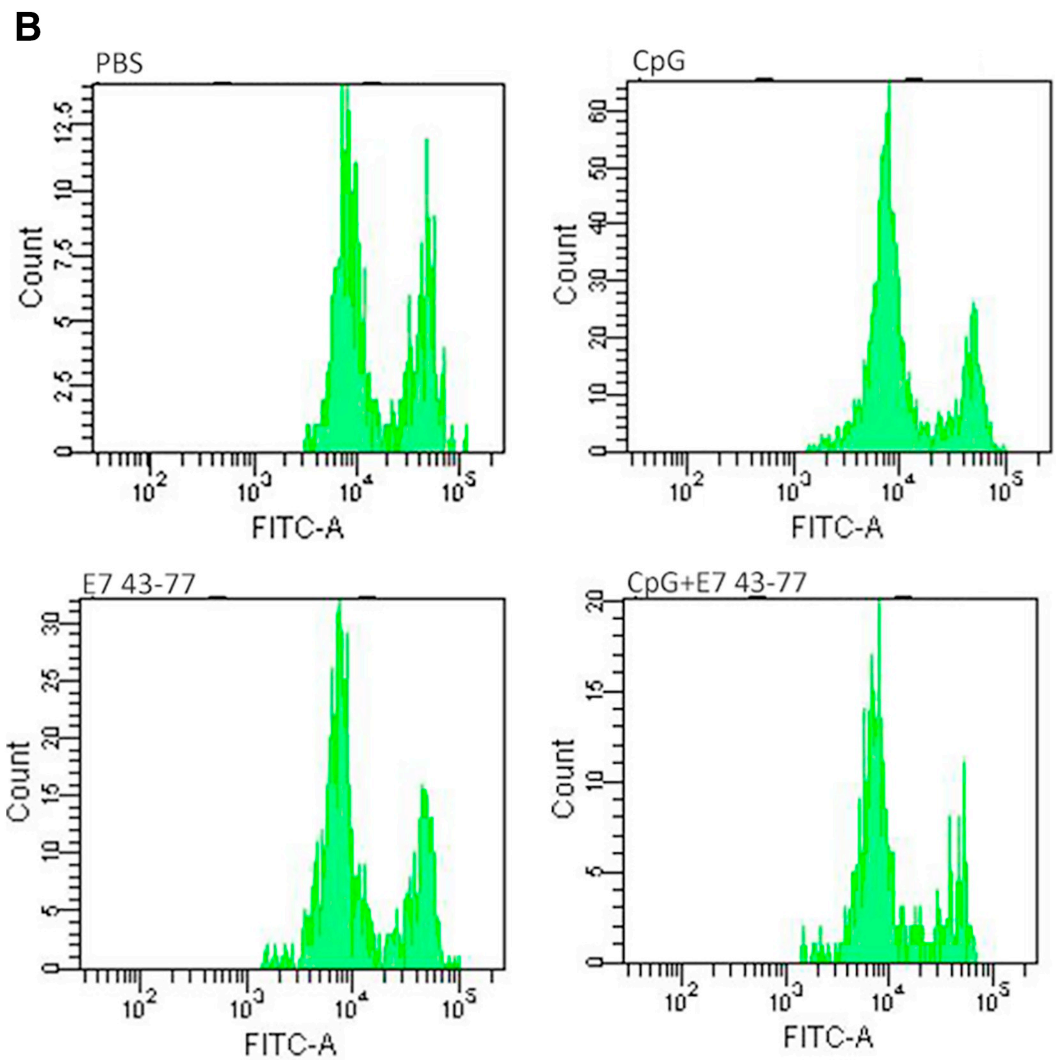


Fig. 3. (*continued*)

3.2. Prevention induced by vaccination

The results of therapeutic effect showed the 50 μg peptide vaccine offered the best therapeutic benefit, so we chose this dose of peptide vaccine for the following prophylactic study. During the course of the prophylactic experiment, none mice inoculated with long peptide vaccine or only 1 mouse inoculated with short peptide vaccine developed tumors which was found from day 19, while all the control mice developed tumors from day 11 and 3 mice inoculated with CpG ODN developed tumors from day 16. All mice in the PBS group had tumors and the average size is 1403.63 mm^3. Half the mice of the CpG ODN group had tumors and the average size is 270.148 mm^3. The results showed vaccine-inoculated mice demonstrated a significantly reduced tumor volume and delayed tumor emergence time compared with the control group (Fig. 2A). Meanwhile, the results of prophylactic effect showed the short peptide vaccine and long peptide vaccine significantly increased tumor-free percentage compared with the control group (Fig. 2B).

3.3. Induction of CTL response by vaccination

The results of therapeutic experiments showed that the long peptide vaccine induced better effect considering complete eradication induced by the long peptide vaccine. Therefore, we explored the CTL response induced by vaccine containing long peptide. The induction of CTL responses is the key therapeutic intervention developed to combat certain types of cancer and intracellular pathogens. In this study, we evaluated CTL response induced by the vaccine *in vivo*. The results showed the three groups vaccinated by CpG alone, long peptide alone and long peptide vaccine induced significant E7-specific CTL responses compared with the control group. In addition, the long peptide vaccine group showed more significantly elevated CTL response than CpG alone and long peptide alone groups (Fig. 3A). Representative results for each group are shown (Fig. 3B).

3.4. Induction of cellular immune response by vaccination

To evaluate the cellular immune response induced by the vaccine, we determined the percentages of total CD4, total CD8, IFN-γ producing, IFN-γ producing CD4, IFN-γ producing CD8 cells in the splenocytes. The results showed that the percentage of total CD4, total CD8, IFN-γ producing, IFN-γ producing CD4 T cells and IFN-γ producing CD8 T cells increased significantly in the long peptide vaccine group compared to PBS group (Fig. 4A, B, C, D, E). Though the percentage also increased in short peptide group, the difference was not statistically significant. Representative dot plots from one mouse (out of three mice) for IFN-γ producing CD4 cells, and IFN-γ producing CD8 cells were shown respectively (Fig. 4F).

3.5. Suppression of immunosuppressive cells induced by the vaccine

To evaluate the systemic immunosuppressive response induced by the vaccine, we determined the percentages of T regulatory cells (CD4 + CD25 + FoxP3 + T cells), macrophages (CD11b + F4/80 + cells), and MDSCs (CD11b + Gr-1 + cells) in the splenocytes. Splenocytes of immunized mice with the long peptide vaccine showed statistically significant decreases in the percentages of CD4 + CD25 + FoxP3 + T cells, macrophages and MDSCs compared with the control (Fig. 5A, B, C). Representative dot plots from one mouse (out of three mice) for CD4 + CD25 + FoxP3 + T cells, CD11b + F4/80 + cells and CD11b + Gr-1 + cells were shown respectively (Fig. 5D, E, F).

4. Discussion

Synthetic peptides derived from HPV 16 E7 were widely applied in therapeutic HPV vaccine against cervical cancer [18,19]. Peptide-based vaccine strategies have several attractive features, such as nontoxic, noninfectious, inexpensive, and relatively easy to produce [20]. To our knowledge, the prophylactic and therapeutic effect on cervical cancer as well as immune responses induced by a single administration of vaccine were comprehensively evaluated in our study for the first time, though it is not the first time to use CpG and E7 deprived peptide against tumor induced by TC-1 cells [21]. And our data demonstrated that in a murine model, the vaccine inhibited the expression of immunosuppressive cells and prevented the tumor formation efficiently.

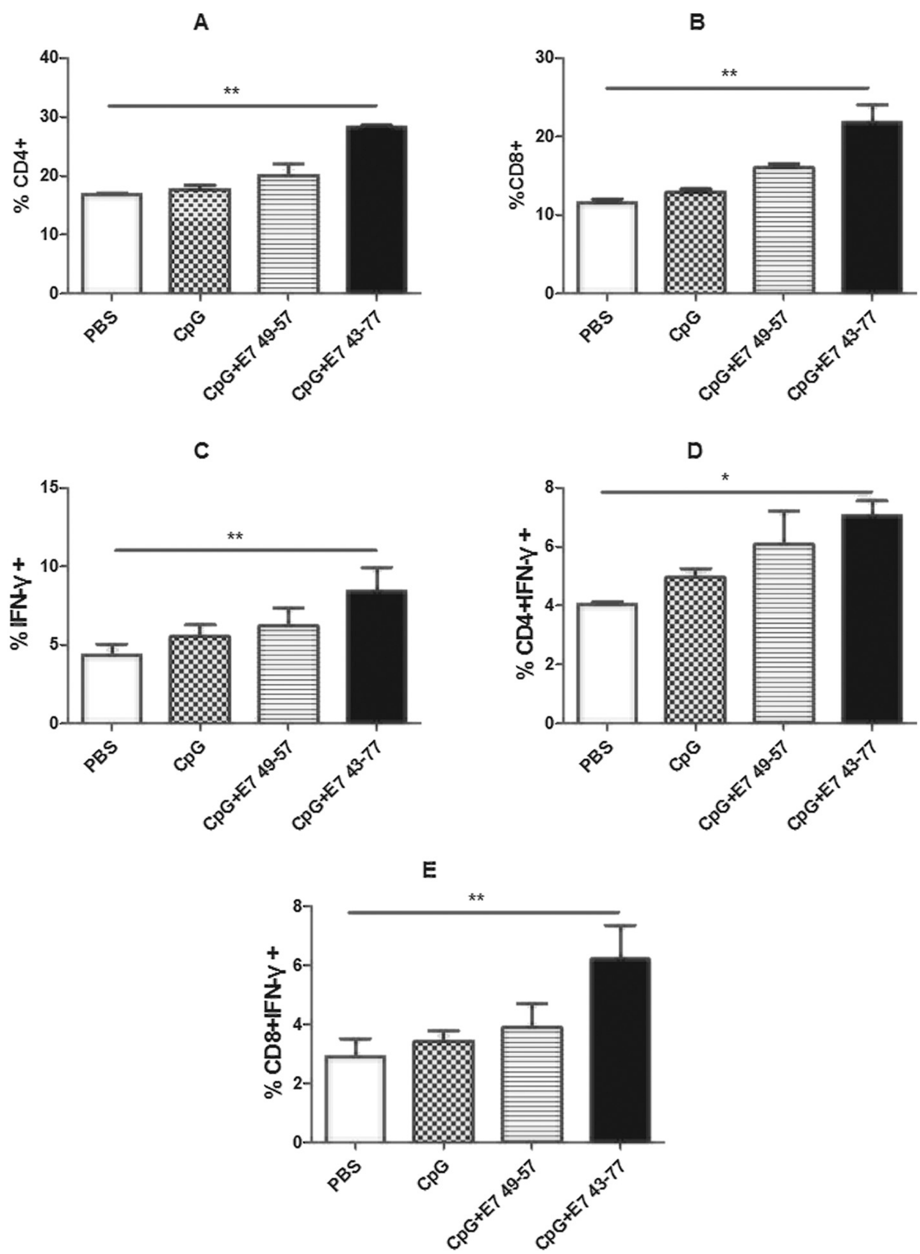
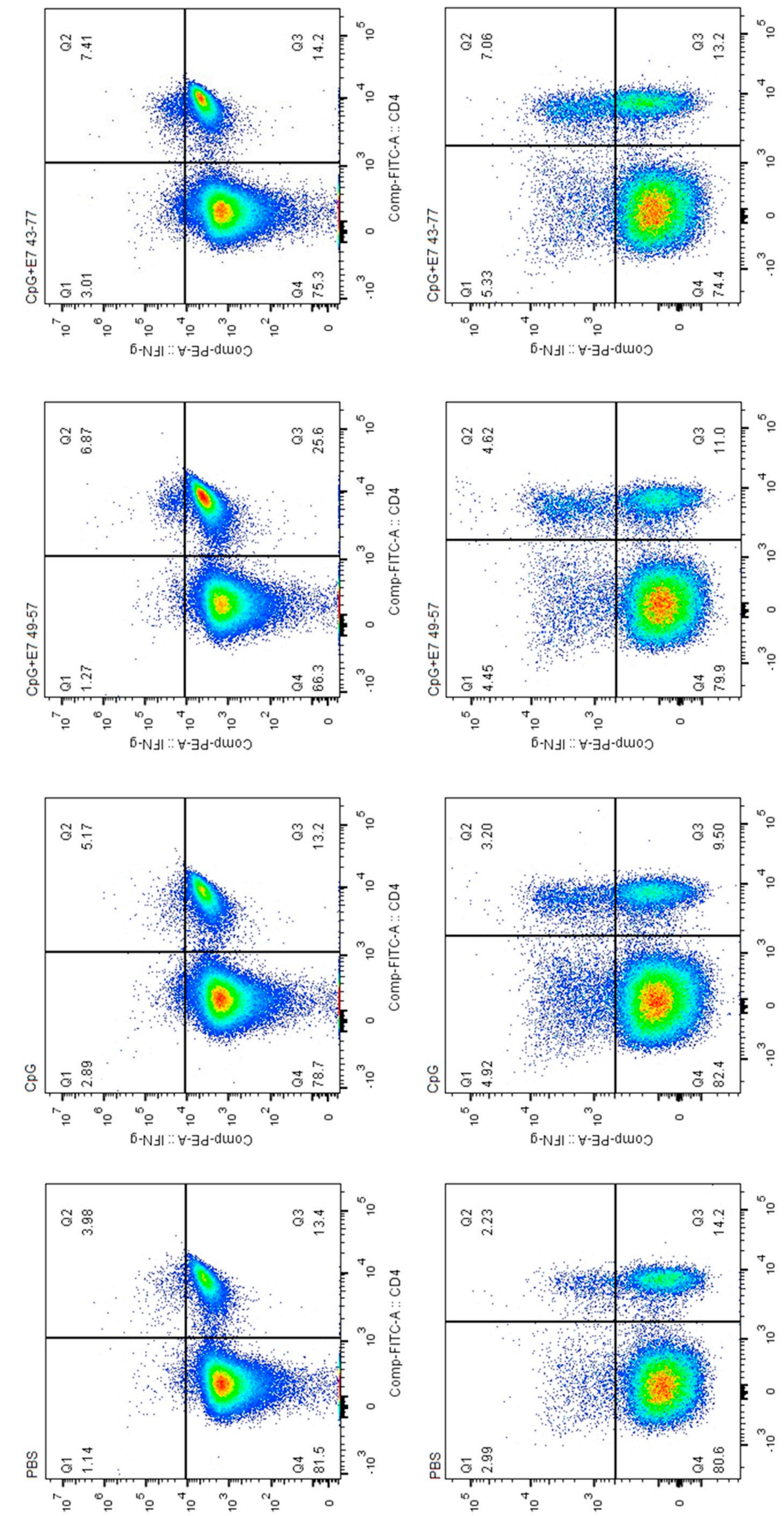


Fig. 4. Cellular immune response induced by the vaccine. Immunized mice ($n = 3$) were sacrificed on day 28. The frequency of total CD4 (A), total CD8 (B), IFN-γ producing cells (C), IFN-γ producing CD4 cells (D) and IFN-γ producing CD8 cells (E) are performed. Representative dot plots from one mouse (out of three mice) for IFN-γ producing CD4 cells, and IFN-γ producing CD8 cells (F) are shown respectively. Data are shown as mean ± s.d. Statistically significant differences (*$P < 0.05$, **$P < 0.01$) are indicated.



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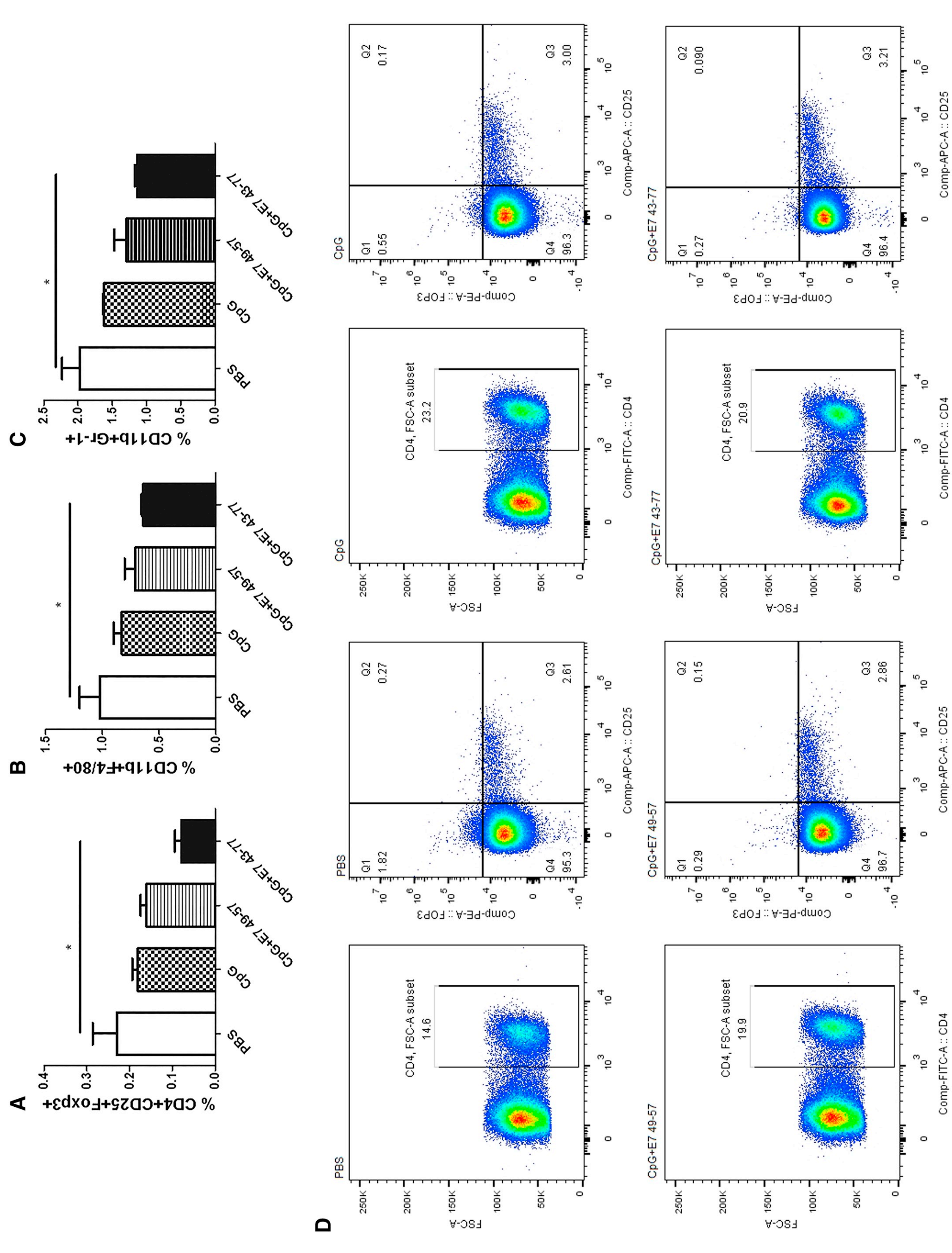

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Fig. 5. Suppression of systemic immunosuppressive cells induced by the vaccine Immunized C57BL mice (n = 3) were sacrificed on day 28. The frequency of CD4 + CD25 + FoxP3+ T cells (A), macrophages (CD11b + F4/80+) (B) and MDSCs (CD11b + Gr-1+) (C) are shown. Representative dot plots from one mouse (out of three mice) for CD4 + CD25 + FoxP3+ T cells (D), CD11b + F4/80+ T cells (E) and CD11b + Gr-1+ T cells (F) are shown respectively. Data are shown as mean ± s.d. Statistically significant differences ($^*P < 0.05$) are indicated.

Cellular immunity plays an important role in clearing HPV-infected cells and HPV-transformed tumor cells, especially CD8+ cytotoxic T cells which have been regarded as major effectors for anti-HPV infection and anti-HPV-induced tumors [22,23]. CD4 T cells play a helping role in priming the generation, expansion, maintenance of CD8 T cells as well as in orchestrating antibody production [24,25]. Moreover, more evidence demonstrated that CD4 T cells contribute to anti-tumor immunity [26,27]. Therefore, simultaneous induction of antigenic-specific CD8 T cells and CD4 T cells by a single vaccine would be more efficacious. An ideal HPV peptide-based vaccine should have capacity of activating antigen-specific CD8 T cells as well as CD4 T cells. Our data showed that the long peptide vaccine induced the increase of total CD4 T cells, total CD8 T cells, IFN-γ-producing cells, IFN-γ producing CD4 T cells, IFN-γ producing CD8 T cells and cytotoxic activity of CD8+ T cells, which may represent one of the mechanisms of the vaccine to eradicate the established tumors.

In this study, the vaccine included peptide HPV16 E7 43-77 as antigen, which contains 1 CD8 T cell epitope (E7 49-57, HLA A24/H-2Db restricted) and 2 CD4 T cell peptide-based vaccines. Short peptide (typically nine amino acid residues)-based vaccine may induce tolerance of antigen-specific CTL activity or inability to reject tumors because short peptides may bind directly to MHC molecules on non-professional antigen-presenting cells (APC) [28]. But for long peptides (> 30-mer), they can't bind directly to MHC on cells and need to be internalized, processed by professional APC for presentation and then induce a more durable adaptive immune response [29]. Moreover, long peptides-based vaccines can induce memory CD8+ T cell responses to enhance tumor control compared with short peptides-based vaccine [30]. In this study, long peptide vaccine induced better prophylactic/therapeutic effect on cervical cancer compared to short peptide vaccine, which may partly result from simultaneous induction of IFN-γ-producing CD8 T cells and CD4 T cells as a result of the incorporation of both CTL and Th epitopes in the long peptide vaccine.

Regulatory T cells (Tregs), a low number of T-cell population, are immunosuppressive cells and generally thought to limit anti-tumor immunity by suppressing the induction and proliferation of effector T cells [31]. They play key roles for maintaining immune system in homeostasis. A balance between the number of Tregs to CTLs in the host is cardinal for having effective immunosurveillance systems [32]. Tregs within the tumor microenvironment have extensive positive interactions with other immunosuppressive cells including cancer-associated fibroblasts, macrophage type 2 cells, and MDSCs, but they have negative interactions with immunostimulatory cells including CTL and NK cells [32]. The number of Tregs is extensively increased in nearly all cancers. High number of Tregs in the tumor microenvironment is indicative of a poor prognosis [33]. Tregs are prevailing cells during neoplastic transformation [32]. Targeting Tregs would bring a benefit for immunotherapy of cancer and responses of cancer cells sensitive to radiation or chemotherapy. Our data showed that the percentage of Tregs decreased significantly after vaccination with long peptide vaccine, which may improve the prophylactic or therapeutic effect of the vaccine. The results also further confirmed Tregs may be targeted for tumor therapy.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expand in some pathological conditions such as cancers [34]. They are thought to contribute to tumor associated immunosuppression *in vivo* by suppressing T and natural killer (NK) cell function [35,36]. MDSCs were also shown to promote the development of Treg cells *in vivo* [37]. Moreover, MDSCs directly interact with tumor cells to support their growth and progression [38–40]. CpG ODN, TLR9 agonist, was reported to be able to induce the expansion of MDSCs in tumor-free mouse. In C26 tumor and autochthonous gastric tumor-bearing mice, CpG ODN treatment didn't increase the number of MDSCs, but promoted maturation and differentiation of MDSCs, significantly decreased the proportion of Ly6Ghi MDSC, and thus blocked the suppressive activity of MDSC on T-cell proliferation [41]. In this study, the number of MDSCs in spleen of vaccinated mice was demonstrated to be decreased which showed the vaccine inhibited the expansion of MDSCs and might revert the immunosuppression in cervical cancer-bearing mice. That might be one of the reasons for a significant eradication of tumor in vaccinated mice. The suppressive activity of MDSCs in vaccinated mice and the exact contribution for the clearance of tumor needed to be investigated in the future.

Macrophages are innate immune cells that play a broad role in host defense and the maintenance of tissue homeostasis [42]. Macrophages shift their functional phenotypes in response to various microenvironmental signals generated from tumor and stromal cells. Several studies reported that TAMs have been shown to provide a microenvironment to promote tumor development and progression. Clinicopathological studies have indicated that patients with higher TAM densities have significantly worse relapse-free survival and overall survival rates [43,44]. Gil-Bernabe AM et al. found that tumor-derived tissue factor (coagulation factor III or CD142) enhanced tumor cell survival at the metastatic site by recruiting CD11b+/CD68+/F4/80+/CX3CR1+ macrophages [45]. Therefore, TAM infiltration appears to be a significant unfavorable prognostic factor for cancer patients, and may be a potentially useful prognostic marker of clinical outcomes. In our study, the number of macrophages in spleen of vaccinated mice was demonstrated to be decreased, which may be involved in the inferred decrease of TAMs recruitment to tumor site and thus favorable tumor microenvironment. Recruitment of TAMs to tumor site and the phenotype of TAMs induced by the vaccine needs to be investigated in the future study though the long peptide vaccine induced decreased percentage of macrophage.

In conclusion, a single administration of HPV peptide vaccine containing E7 43-77 and CpG ODN as an adjuvant elicited significant prophylactic and therapeutic effect on cervical cancer. The vaccine induced increased cellular immunity and decreased numbers of immunosuppressive cells including MDSCs and Tregs, which may result in the satisfied prophylactic and therapeutic effect of the vaccine on cervical cancer.

Author contributions statement

Study concept and design: Wang Xue-lian. Acquisition of data: Yang Yang, Che Yu-xin and Zhao Yan. Analysis and interpretation of data: Yang Yang, Che Yu-xin, Zhao Yan and Wang Xue-lian. All authors were involved in drafting and critical revision of the manuscript. All authors approved the final version.

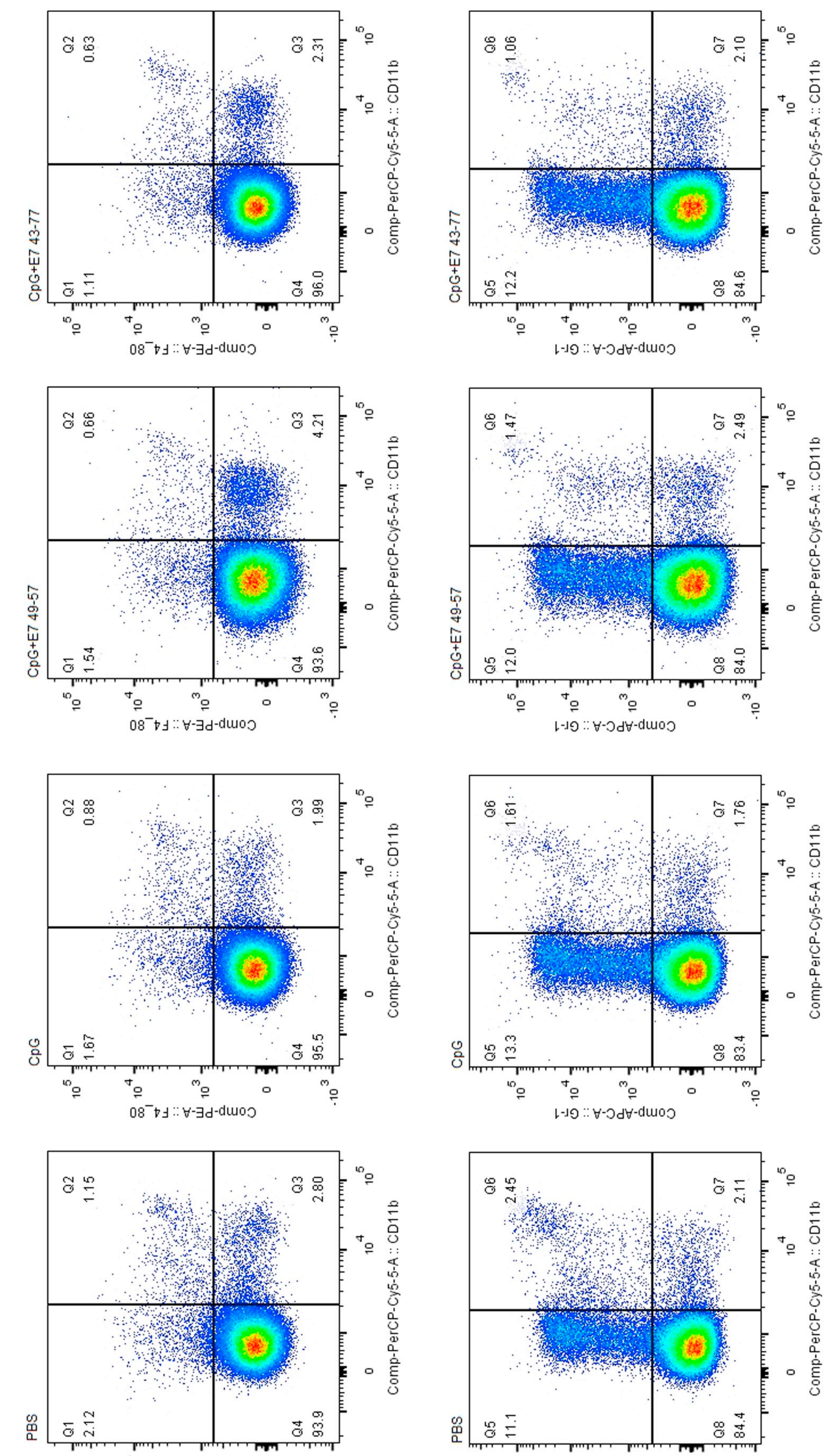

Fig. 5. (*continued*)

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81472439), the Liaoning Provincial Natural Science Foundation of China (No. 20180550760) and the Scientific Research Fund of Liaoning Provincial Education Department (No. L2010709).

Conflicts of interest statement

All authors have no conflicts of interest to declare.

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