Arch Dermatol Res (2010) 302:57–65
DOI 10.1007/s00403-009-0976-0

ORIGINAL PAPER

Toll like receptor agonists augment HPV 11 E7-specific T cell responses by modulating monocyte-derived dendritic cells

Xian-Zhen Chen · Xiao-Hong Mao · Ke-Jian Zhu · Na Jin · Jun Ye · Jian-Ping Cen · Qiang Zhou · Hao Cheng

Received: 6 February 2009 / Revised: 12 June 2009 / Accepted: 19 June 2009 / Published online: 4 July 2009

Abstract Impaired local cellular immunity is one of the mechanisms responsible for condyloma acuminatum (CA) recurrence. The activation of dendritic cells (DCs) is important in vaccine development. We investigated the effect of different toll like receptor (TLR) agonists including LPS (TLR4 agonist), polyinosinic acid-polycytidylic acid (PIC, TLR3 agonist), CpG oligonucleotide (TLR9 agonist), and imiquimod (TLR7 agonist) on human monocyte-derived dendritic cells (mdDCs) loading of human papillomavirus (HPV) type 11 E7 epitope. As a result, we found that mdDCs loading HLA-A*0201-restricted HPV 11 E7 CTL epitope peptide could respond to the TLR agonists, especially LPS and PIC. This was characterized by an enhanced expression of CD40, CD80, CD86, CD83 and HLA-DR, and a high level of IL-12 production. TLR agonists, especially PIC, enhanced the ability of E7-loaded mdDCs to induce IFN-γ-secretion $CD4^+$ naïve T cells. Moreover, E7-loaded mdDCs exposed to TLR agonists augmented autologous T cell responses including effector cytokines production and specific cytotoxic T lymphocyte (CTL) responses. In addition, the inhibitory effect of IL-10 on mdDCs maturation could be partially restored by LPS, PIC or imiquimod. Taken together, these results demonstrate that TLR agonists promoted the maturation of E7-loaded mdDCs and their ability to induce T help type 1 polarization and augment E7-specific T cell responses. These data also indicated that TLR3/4 agonists might be effective adjuvants of mdDC-based vaccines against CA.

Keywords Human papillomavirus · Condyloma acuminatum · Toll like receptor · Agonist · Dendritic cells

X.-Z. Chen · K.-J. Zhu · N. Jin · J. Ye · J.-P. Cen · Q. Zhou · H. Cheng (✉)
Department of Dermatology and Venereology, Biomedical Research Center, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, 3 East Qingchun Road, 310016 Hangzhou, Zhejiang, People's Republic of China
e-mail: hz_chenghao@126.com

X.-H. Mao
Department of Dermatology and Venereology, School of Medicine, The Second Affiliated Hospital, Zhejiang University, 88 Jiefang Road, 310009 Hangzhou, Zhejiang, People's Republic of China

Introduction

Condyloma acuminatum (CA), one of the most common sexually transmitted diseases caused by human papillomavirus (HPV), is characterized by high recurrence. The persistent infection of HPV is considered to be a major epidemiological marker of individual risk for anal or cervical cancers [12, 17, 21, 26]. CA-related emotional distress and heavy economic burdens have been noticed. Therefore, a strategy to effectively manage CA would have far-reaching global health and economic impact.

Evidences have shown that the local cell-mediated immunity was suppressed in CA lesions [30], especially in the patients with recurrent CA [5, 14, 36, 38, 40]. Langerhans cells (LCs) or dermal dendritic cells (DCs) are the most important antigen-presenting cells in skin [11]. However, the number and the function of LCs are significantly reduced in CA lesions [27, 29]. This might attribute to the insufficient induction of a more threatening T help type 1 (Th1) response which would favor the development of cytotoxic T lymphocytes (CTLs). Additionally, the down-regulation of TNF-α and the up-regulation of the immunosuppressive cytokine IL-10 in CA lesions may

subsequently suppress the anti-viral activities of the Th1 cells [2, 29].

Antigen-based therapeutic HPV vaccines are proved to potentially eliminate pre-existing lesions and malignant tumors by generating cellular immunity against HPV-infected cells [22]. The key point of these vaccine strategies is to elicit the necessary processing and presenting of peptide, and subsequently the enhanced activation of T cells [16, 18, 41]. In this regard, the delivery of ex vivo pre-treated DCs may be an alternative [1, 32]. There are several studies on DC vaccine against high-risk HPV related cervical carcinoma [32]. In clinical trials, autologous DCs pulsed with HPV 16/18 E7 protein can induce T cell responses in a portion of both early and late stage cervical cancer patients, and finally lead to a slow tumor progression [15, 33]. Therefore, therapeutic DC vaccine such as HPV 11 E7 protein-pulsed DCs might be a potential approach to manage the low-risk HPV infection such as CA. On this basis, boosting of immune responses by effective adjuvants will benefit the vaccination.

Toll like receptors (TLRs) recognize motifs of microbial pathogens for early recognition of microbial invasion. Their ligation by microbial elements is critical in DC activation and maturation, and therefore important for the adaptive pathogen-specific T cell response [7, 23, 25]. To analyze the substantial effect of TLR agonists on adjuvants of a HPV epitope peptide vaccine regimen, we studied the ability of various TLR agonists to modulate the maturation of HPV 11 E7 CTL epitope peptide-loaded mdDCs, and further to induce HPV-specific Th1 and $CD8^+$ T cell responses. MdDCs may represent immature dendritic cells which home at sites of inflammation [4]. Moreover, the availability of large numbers differentiated mdDCs facilitated the study to compare several TLR agonists side by side.

Materials and methods

Isolation of monocytes, T cells, and naïve T cells

Peripheral blood mononuclear cells (PBMC) were isolated from HLA-A*0201 healthy donors by Lympholyte-H (Cedarlane Laboratories, Canada) density gradient centrifugation as recommended by the manufacturer. CD14 positive monocytes were isolated with paramagnetic beads (MiltenyiBiotec) as previously described [39]. The purity of the cell separation tested by FACS was 95.3% (±4.1%).

T cells were isolated with pan T cell isolation kit (MiltenyiBiotec) from the remaining monocyte-depleted cells [39]. $CD4^+$ T cells were then collected after depletion of $CD8^+$ T cells with anti-CD8 paramagnetic beads (MiltenyiBiotec). Afterwards, $CD4^+CD45RA^+$ naïve T cells were labeled with anti-CD45RA paramagnetic beads (MiltenyiBiotec) and enriched on separation columns in magnetic field (MidiMACS™, MiltenyiBiotec). All the cells were incubated in the standard medium [RPMI 1640 (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/l L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin].

Generation and stimulation of mdDCs

Immature dendritic cells (imDC) were generated from $CD14^+$ cells as previously described [39]. On day 6, the CD1a expression of the cultured cells was 61.2% (±11.36%). 2×10^6 differentiated DCs were then co-cultured with immunodominant HLA-A*0201-restricted HPV 11 E7 CTL epitope peptide (TLKDIVLDL) screened previously [39] in six-well plate (20 µg/ml) for 1–12 h. Cultures were then supplemented with different TLR agonists for 48 h at the following concentrations: LPS from *Escherichia coli* 0111:B4 strain (TLR4 agonist, Sigma, USA) 0.4 µg/ml, imiquimod (TLR7 agonist, Mingxin Pharmaceutical Ltd, China. Imiquimod was dissolved in 0.01 M HCl solution to obtain a concentration of 0.50 mg/ml) 4 µg/ml, polyinosinic acid: polycytidylic acid (PIC, TLR3 agonist, InvivoGen, USA) 20 µg/ml, CpG oligonucleotide 1826 (5′-TCCATCACGTTCCTGACGTT-3′, TLR9 agonist, Invitrogen, USA) 10 µg/ml, or a combination of PIC, imiquimod and CpG. The CD1a expression of the stimulated cells was 97.1% (±5.23%). The concentration of all TLR agonists were determined in preliminary experiments (data not shown), at which the agonists induced significant DC phenotypic differentiation without cell death. Additionally, exogenous human IL-10 (50 ng/ml medium, Peprotech Inc., USA) was added to cultures together with various TLR agonists during the DC maturation in an independent experiment.

Phenotyping of mdDCs

MdDCs were characterized on day 6 (immature mdDCs) and day 8 (mature mdDCs) using following fluorochrome-labeled monoclonal antibodies (anti-CD1a, anti-CD40, anti-CD80, anti-CD83, anti-CD86, anti-HLA-DR, as well as their corresponding PE- or FITC-labeled isotype control antibodies; eBioscience, USA) and analyzed on an EPICS-XL flow cytometer (Beckman Coulter, USA).

Cytokine production of mdDCs by stimulation with E7 peptide and TLR agonists

Supernatants were collected from cultures of immature, unstimulated, or stimulated mdDCs. Secretion of IL-12p70, IL-10, and IFN-α was measured with corresponding ELISA

kits according to the manufacturer's instructions (Biosourse, USA).

Th1/Th2 polarization

Isolated naïve T cells (2.5×10^5 cells/500 µl medium) were co-cultured with 5×10^4 HPV 11 E7-loaded mdDCs or various TLR agonist-pretreated mdDCs in 24-well plates for 6 days. Supernatants were collected, and cytokine (IFN-γ and IL-4) concentration was measured by ELISA according to the manufacturer's instructions (Biosourse, USA).

Cytokine production of T cells cultured with mdDCs

The HPV 11 E7 CTL epitope peptide-loaded or various TLR agonist-pretreated mdDCs (2×10^5 cells/2 ml medium) as described were co-cultured with 2×10^6 T cells in six-well plates at 1-week interval for two times. T cell culture supernatants were analyzed for IFN-γ, TNF-α, and IL-2 with the corresponding ELISA Kits (Biosourse, USA). All culture supernatants were kept at −20°C until use.

Enzyme-linked immunospot (ELISPOT) assay for IFN-γ and IL-2 production

E7 peptide-loaded mdDCs, various TLR agonist-stimulated mdDCs, and E7 peptide plus various TLR agonist-stimulated mdDCs were used as stimulator cells. T cells (1×10^5) and stimulator cells (2×10^4) were seeded into 96-well polyvinylidene fluoride (PVDF)-backed microplates coated with monoclonal antibody specific for human IFN-γ or IL-2 (R&D Systems, USA). After incubation at 37°C for 24 h, the cells were removed and the plates were processed according to the manufacturer's instructions. Resulting spots in each well were counted with an ELISPOT Reader (Cellular technology Ltd, USA) and analyzed with the ImmunoSpot 4.0 software. The values were expressed as spot-forming cells (SFCs) per 10^6 T cells.

Specific CTL assay

Purified $CD8^+$ T cells primed with HPV 11 E7 epitope peptide-loaded, TLR agonist-pretreated mdDCs served as effector cells. The HPV11E7-expressing human embryonic kidney (HEK) 293 cells established previously [39] were used as target cells. Briefly, 5×10^3 target cells were cocultured with effector cells in effector-to-target cell (E/T) ratios of 100:1, 50:1 and 20:1. $CD8^+$ T cells primed by mdDCs without peptide loading or by E7-mdDCs unstimulated with TLR agonists were served as controls. After 6 h of incubation at 37°C, the supernatant was collected to assess the lactate dehydrogenase concentration using CytoTox 96 nonradioactive cytotoxicity assay kits (Promega Corp, Madison, USA). The cytotoxicity activity of T cells was assessed based on the following formula with the mean values from quadruple wells: Percent cytotoxicity = (experimental value − effector spontaneous value − target spontaneous value)/(target maximum − target spontaneous value) × 100 [9].

Statistical analysis

All data were expressed as mean ± SD. One-way ANOVA was used to evaluate the significance of group differences. Values of $P < 0.05$ were considered to be statistically significant.

Results

Phenotypic maturation of mdDCs by TLR agonists' stimulation

LPS and PIC induced a significant up-regulation of CD40, CD80, CD83, CD86, and HLA-DR expression on mdDCs as compared with medium alone ($P < 0.05$) (Fig. 1a). LPS and PIC induced a significantly higher expression of CD40, CD83, and CD86 on mdDCs as compared with imiquimod ($P < 0.05$) or CpG ODN (all $P < 0.01$) (Fig. 1a). Imiquimod induced higher expression of CD80, CD83, and CD86 on mdDCs than that of control mdDCs, except for the expression of CD40 and HLA-DR ($P \geq 0.05$). No increased expression of CD40, CD80, CD86, and HLA-DR on CpG ODN-stimulated mdDCs was observed ($P \geq 0.05$).

The CD40, CD83, and CD86 expression of the mdDCs stimulated with IL-10 was lower than that of the non-stimulated mdDCs, while the CD80 and HLA-DR expression was comparatively stable (Fig. 1b). The expression of CD83, CD86, and HLA-DR on mdDCs stimulated with IL-10 plus LPS, PIC or imiquimod stimulation was higher than that of mdDCs stimulated with IL-10 alone (Fig. 1b).

Cytokine production of mdDCs by HPV11E7 plus TLR agonists' stimulation

We investigated the secretion of IL-12 p70 and IFN-α, two potential Th1-inducing cytokines, and IL-10 by HPV 11 E7 epitope peptide-loaded mdDCs in response to different TLR ligation. High level of IL-12 was produced by mdDCs stimulated with either LPS or PIC (2155.7 ± 133.5 and 1971.7 ± 144.8 pg/ml, respectively) ($P < 0.01$, compared with control mdDCs or mdDCs loading of HPV 11 E7 epitope peptide only) (Fig. 2). Imiquimod and CpG induced higher level of IL-12 by mdDCs (495.5 ± 132.7 and 484.3 ± 140.0 pg/ml, respectively) than that of the control group ($P < 0.05$) but much lower than that of LPS or PIC group ($P < 0.01$) (Fig. 2). IFN-α was poorly produced by

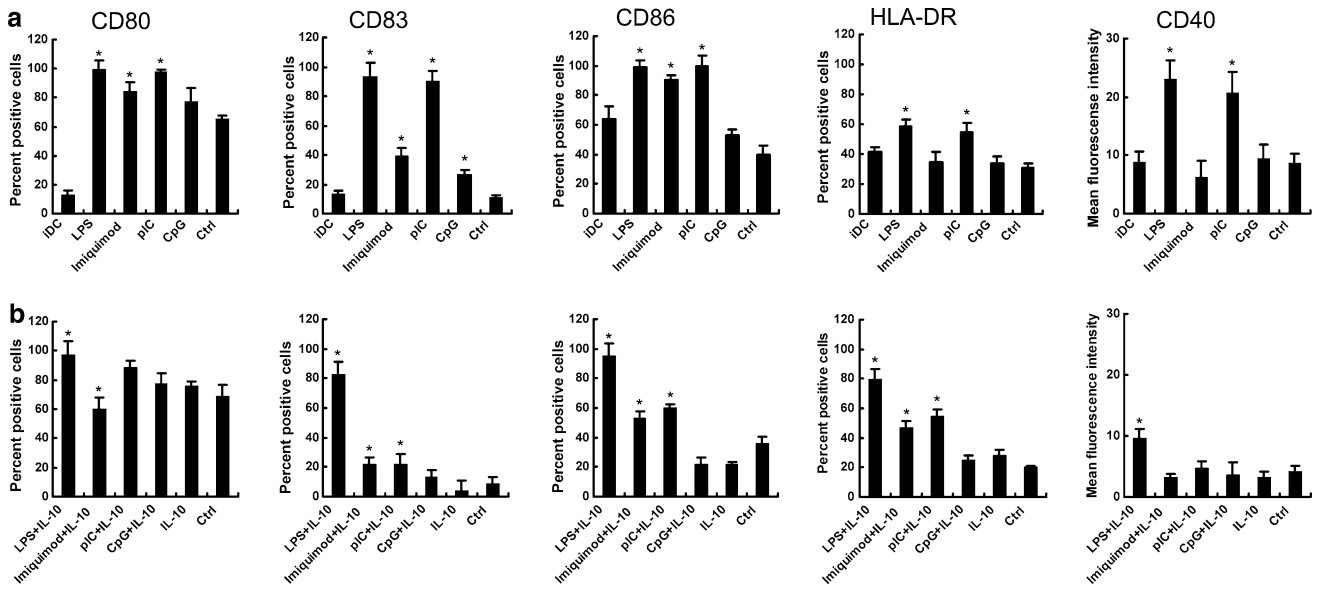

Fig. 1 MdDCs differentiate in response to TLR ligation and/or IL-10 after culture for 48 h. Surface expression of various markers (CD40, CD80, CD86, CD83, and HLA-DR) of mdDCs after exposure to indicated TLR agonists (**a**) or to TLR agonists plus IL-10 (**b**) were measured by flow cytometry. Data presented are mean ± SD values of from five donors. *Indicates statistical significance ($P < 0.05$) when compared with the control group

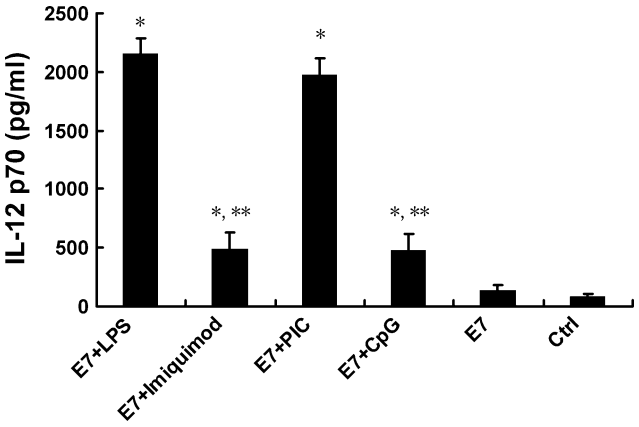

Fig. 2 IL-12 p70 production by mdDCs stimulated with various TLR agonists for 48 h. Supernatants were assessed for IL-12 production by ELISA. Results shown represent the mean ± SD from five donors. *Indicates statistical significance ($P < 0.05$) when compared with the control group. **Indicates statistical significance ($P < 0.05$) when compared with the LPS group

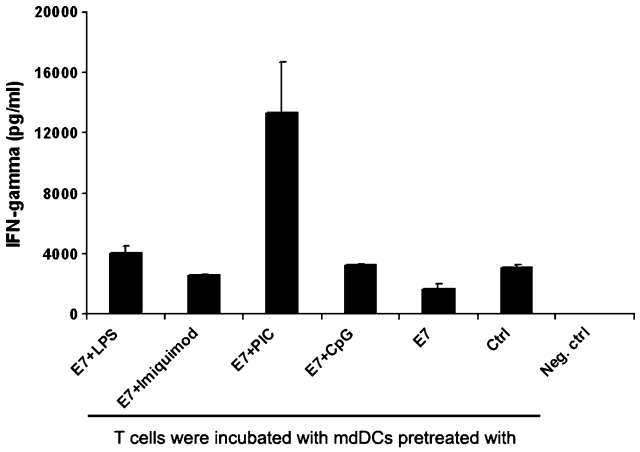

Fig. 3 Effect of TLR agonists on cytokine production by $CD4^+$ Th cells. Allogenic $CD4^+CD45RA^+$ T cells were co-cultured for 6 days with effector mdDCs pretreated with the indicated TLR agonists. The release of IFN-γ and IL-4 (not shown in the figure) was measured by ELISA. Results are representative of three independent experiments

mdDCs exposed to the studied TLR agonists except the mdDCs stimulated with LPS (2045.6 ± 389.2 pg/ml). IL-10 was undetectable by all the TLR agonist-stimulated mdDCs except LPS-stimulated mdDCs (1089.7 ± 264.0 pg/ml).

TLR agonist pre-treated mdDCs enhanced $CD4^+$ naïve T cell differentiation

Th1 and Th2 development was assessed by quantifying IFN-γ and IL-4 secreted by $CD4^+$ naïve T cell. As shown in Fig. 3, no IFN-γ secretion was detected from the unstimulated naïve T cells. And the IFN-γ secretion of naïve T cells was increased by HPV 11 E7 epitope peptide-loaded mdDCs stimulated with all TLR agonists. Among these agonists, PIC-treated mdDCs stimulated naïve T cells to produce IFN-γ nearly twofold higher than that of LPS, imiquimod or CpG groups. On the contrary, IL-4 was not detectable in each group (the test range of ELISA kit is from 0 to 500 pg/ml).

Cytokine production of T cells induced by mdDCs pretreated with various TLR agonists

T cells stimulated with HPV 11 E7 epitope peptide-loaded mdDCs which were subsequently stimulated with LPS or PIC, but not imiquimod or CpG ODN, secreted strikingly higher levels of IFN-γ (Fig. 4a), TNF-α (Fig. 4b), and IL-2 (Fig. 4c), compared with mdDCs loaded with E7 only. IFN-γ, TNF-α, and IL-2 production of the T cells stimulated with mdDCs which were pretreated with a combination of PIC, imiquimod, and CpG ODN was not higher than that of the T cells stimulated with the PIC-pretreated mdDCs (Fig. 4).

The frequencies of IFN-γ and IL-2-producing T cells after stimulation with various TLR agonists pretreated mdDCs

As shown in Fig. 5, mdDCs pretreated with HPV 11 E7 epitope peptide plus LPS or PIC could induce more IFN-γ- and IL-2-secreting cells than irrelevant peptide pulsing mdDCs or PBS ($P < 0.05$). MdDCs stimulated with E7 epitope peptide plus LPS or PIC, but not plus imiquimod or CpG ODN, had significantly higher frequencies of IFN-γ- (Fig. 5a) and IL-2- (Fig. 5b) producing cells when compared to mdDCs stimulated with E7 epitope peptide alone or E7 plus IL-10 (all $P < 0.01$). There was no difference between the frequencies of IFN-γ- (Fig. 5a) and IL-2- (Fig. 5b) producing cells elicited by mdDCs pretreated with TLR agonists plus E7 and mdDCs pretreated with various TLR agonists alone (all $P > 0.10$).

Cytotoxicity of the E7 epitope peptide-specific $CD8^+$T cells towards E7-expressing cells

The E7 epitope peptide-specific $CD8^+$ T cells primed by all TLR agonist-pretreated mdDCs could induce lysis of E7-expressing cells. LPS- or PIC-pretreated mdDCs induced a strong CTL activity (83.1 and 81.3%, respectively) in an effector/target cell (E/T) ratio of 100:1, that is much more efficient than that of mdDCs stimulated with E7 epitope peptide only (56.5% cytotoxicity), mdDCs stimulated with non-sense peptide (19.7% cytotoxicity), and mdDCs unstimulated (16.1% cytotoxicity) (Fig. 6). The $CD8^+$ T cells primed by imiquimod or CpG-pretreated mdDCs exhibited 62.2 and 59.7% cytotoxicity, respectively, in an E/T ratio of 100:1. No difference was observed when compared with mdDCs stimulated with E7 only. The CTL activities in the LPS, PIC, imiquimod, and CPG ODN group were above 50% in the E/T ratio of 50:1.

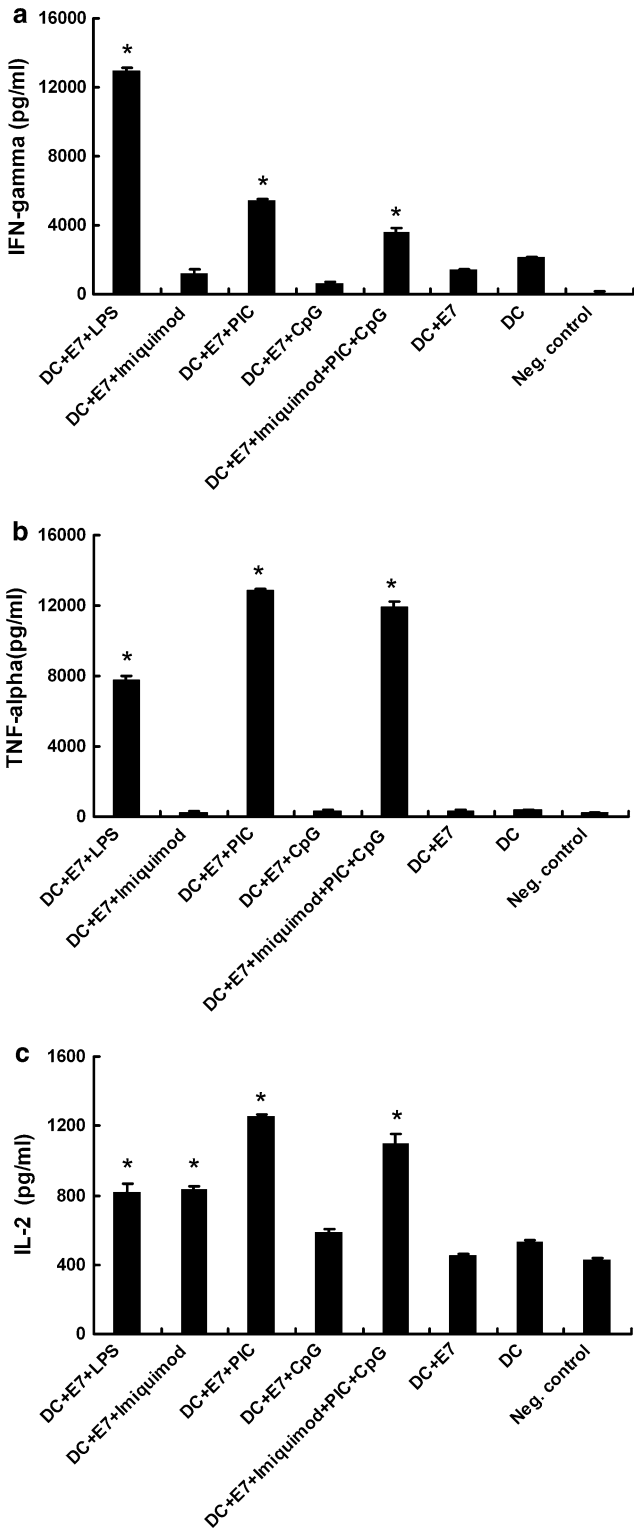

Fig. 4 Cytokine production of IFN-γ, TNF-α and IL-2 by T cells in response to TLR agonists and HPV 11 E7 epitope peptide-pretreated mdDCs. Allogenic T cells were co-cultured with 2×10^5 indicated mature mdDCs. Cytokine production from each culture was measured after 14 days by ELISA. Results shown are means $\pm$ SD of three independent experiments

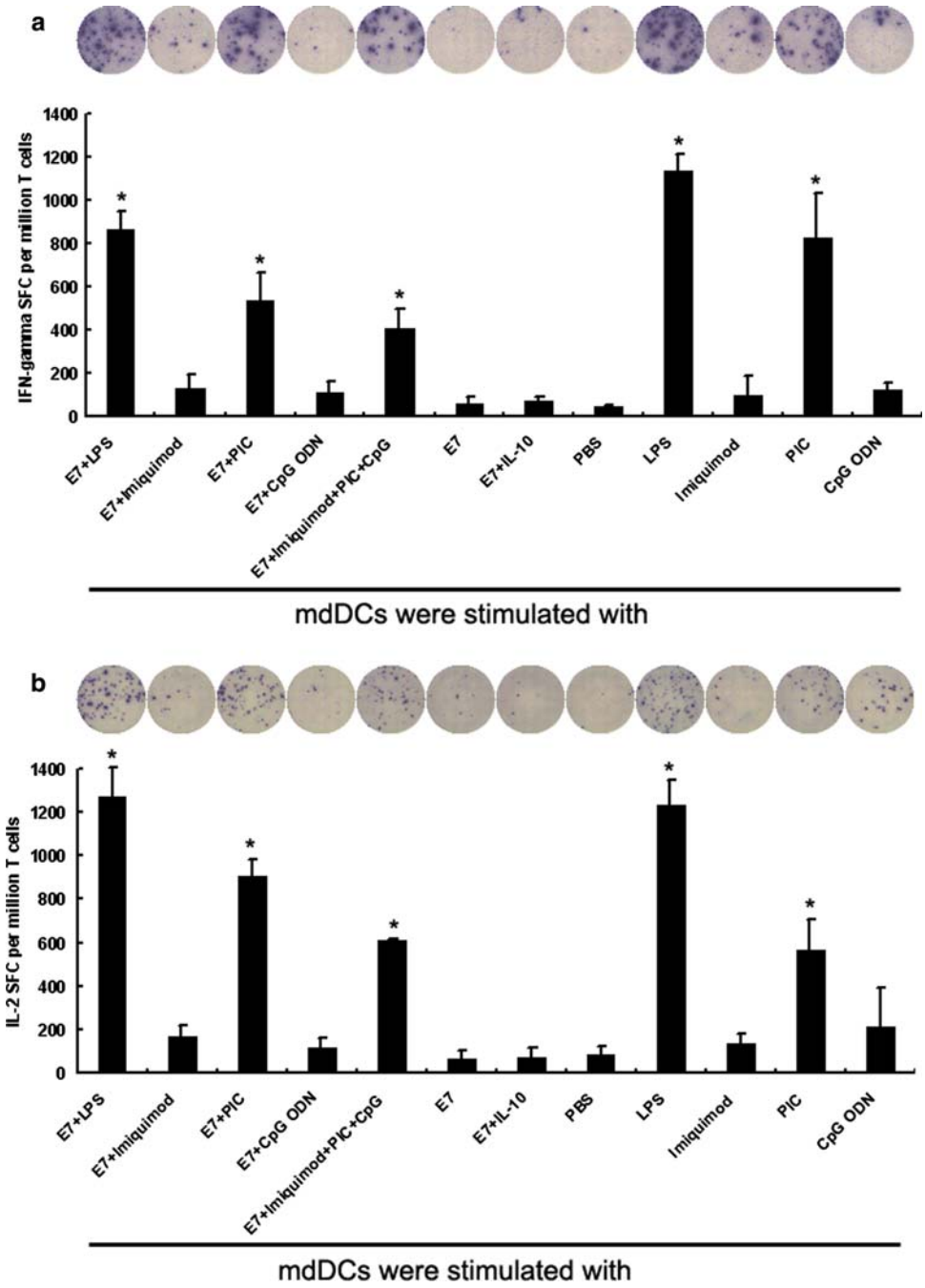

Fig. 5 IFN-γ and IL-2 ELISPOT assays. 1×10^5 purified allogenic T cells were cocultured with 2×10^4 the indicated effector mdDCs in vitro, and the frequency of IFN-γ or IL-2 secreting cells was assayed with the human IFN-γ/IL-2 ELISPOT kit according to the manufacturer's instructions. The values are expressed as spot-forming cells (SFCs) per 10^6 T cells. The data are the mean $\pm$ SD of triplicate values

Discussion

In order to evaluate the immunostimulatory effects of various TLR agonists upon mdDCs loading immunodominant CTL epitope peptide of HPV 11 E7, we assessed the phenotypic maturation and the cytokine production of mdDCs, as well as their ability to augment T cell responses including cytokine production and specific CTL activity.

MdDCs maturation is an important step in their functional capacity to activate T cells. The phenotypic maturation of mdDCs after exposure to various TLR agonists was characterized in the current experiment. MdDCs' maturation in response to PIC and LPS was demonstrated by significant up-regulation of the costimulatory molecules CD40, CD80, CD86, maturation-associated marker CD83, and MHC class II (HLA-DR), while imiquimod and CpG induced less, but still notable phenotypic changes. It indicated that TLR agonists, especially PIC and LPS, could induce the maturation of mdDCs and might be severed as alternative maturation stimulus of mdDCs apart from the classical approaches.

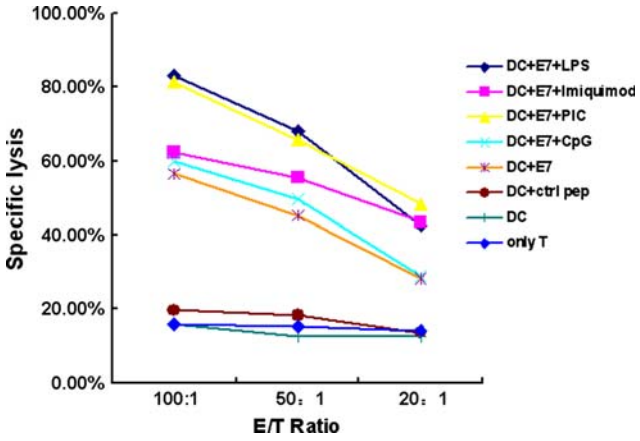

Fig. 6 Cytotoxicity of the HPV 11 E7 epitope peptide specific $CD8^+$ T cells against HPV 11 E7/B16 cells. $CD8^+$ T cells were pretreated with peptide (HPV 11 E7 or non-sense pepitide) pulsed mdDCs stimulated or unstimulated with TLR agonists. The cytotoxicity against target cells was determined by the release of LDH after 6 h of co-culture at different effector/target cell ratio. Percentage of cytotoxicity was calculated as described. The result is a representation of three independent experiments

A serious disadvantage of current ex vivo generated human mdDC is the poor production of IL-12p70 which is important for the type 1 polarization of T cell immunity [3, 35]. Prior studies showed that immature mdDCs produced high levels of IL-12 by TLR3, TLR4, TLR7/8, or TLR9 ligand stimulation [13, 24]. MdDCs matured with LPS, PIC, and/or R848 (a much more potent ligand of TLR7/8 than imiquimod) [37] were able to produce vast amounts of IL-12p70 [6]. TLRs thereby could be effective targets for enhancing IL-12 production of mdDCs. In accordance with these studies, we found that mdDCs were much more susceptible to PIC or LPS stimulation than imiquimod or CpG ODN stimulation in respect of IL-12 secretion. However, IFN-α, which can orient DC functions towards the priming and expansion of protective antiviral immune responses [34], was not detectable in the TLR-activated mdDCs except for LPS stimulation. This might be explained by the low level of IFN regulatory factor (IRF)-7 expression in immature mdDCs [10, 19], whereas the IFN-α production induced by LPS might be largely dependent on its ligation with the remaining CD14 (another receptor of LPS) expressing on the mdDCs rather than with TLR4. Therefore, of the cytokines measured, IL-12 p70 is the predominant cytokine upregulated by TLR agonists in activated mdDCs.

IL-10, which increased in CA lesion [2, 29], was reported to impair DC function by holding the DCs in immaturity [20, 28]. The apparent inhibition of IL-10 on mdDCs' maturation in this study is consistent with some previous studies [8]. We also found that LPS- or PIC-induced maturation of mdDCs was less suppressed by IL-10 as compared to imiquimod and CpG ODN. It suggests that the mdDCs' maturation in the circumstance of increased IL-10, such as in CA lesions, could be partially restored by LPS or PIC.

The increased production of IL-12 and the decreased production of IL-10 would be predicted to affect Th effector cell development. By co-culturing various TLR agonist-pretreated effector DCs with naive Th cells, we delineated the ability of these agonist-treated DCs to regulate specific subset differentiation of $CD4^+$ T cells. Evaluation of cytokine production profiles revealed that after stimulation with TLR agonists, E7-loaded mdDCs stimulated naïve CD4 $\pm$ T cells to produce high level of IFN-γ. PIC-pretreated mdDCs were much more effective in stimulating naïve T cells to produce IFN-γ than LPS-, imiquimod- or CpG ODN- pretreated mdDCs. However, the Th2 type cytokine, IL-4, was undetectable. It indicates that PIC-pretreated mdDCs, could strikingly induce the Th1 response and will favor the production of CTL effectors which are important in clearing virally infected cells.

We also found that TLR ligand-induced activation of mdDCs significantly enhanced production of cytokines (IFN-γ, TNF-α and IL-2) and accordingly increased frequencies of activated T cells. The PIC was the most effective stimuli for mdDCs as compared to the positive control LPS. Importantly, $CD8^+$ T cells primed by LPS- and PIC-pretreated mdDCs loading of HPV 11 E7 epitope peptide strikingly increased specific CTL activity against HPV 11 E7-expressing cells as compared to T cells primed by mdDCs stimulated with imiquimod or CpG ODN, mdDCs loading of HPV 11 E7 epitope peptide, or mdDCs loading of non-sense epitope. This suggests that E7-specific antiviral T cell immunity can be mostly augmented through LPS or PIC-modulated mdDCs. Imiquimod and CpG ODN were relatively poor adjuvants for eliciting T cell responses against HPV 11 E7-expressing cells.

Renn et al. [31] analyzed the TLR 1-10 expression in mdDCs by real-time PCR. They found that mdDCs expressed mRNAs for TLR 1-10 with high expression of TLR2 mRNA, intermediate expression of TLR8, TLR4, TLR3, and TLR10 mRNAs, and low expression of TLR1, TLR5, TLR6, TLR7, and TLR9 mRNAs. It was reasonable that in the current study, the activation of mdDCs by the TLR3 agonist PIC and the TLR4 agonist LPS was much higher than the activation induced by TLR7 agonist imiquimod, and TLR9 agonist CpG ODN. Therefore, LPS and PIC might be more promising adjuvants, than imiquimod and CpG ODN, in mdDC-based vaccine strategies against CA.

In summary, our study provides a reference of using predominant CTL epitope of HPV 11 E7 and TLR agonist immunization for eliciting specific cellular immune responses. These data might favor the rational design of TLR-based therapeutic vaccines or immune-modulating therapy against CA. Further investigation in mouse model expressing HPV 11 E7 is under research now.

Acknowledgments This research was supported by Science and technology Foundation of Zhejiang province (2008C23046) and Creative Funds from Health Bureau of Zhejiang Province (491030-710901). We are grateful to Xunzi Cai for critical review of the manuscript.

References

1. Adams M, Borysiewicz L, Fiander A et al (2001) Clinical studies of human papilloma vaccines in pre-invasive and invasive cancer. Vaccine 19:2549–2556
2. Arany I, Tyring SK (1996) Status of local cellular immunity in interferon-responsive and -nonresponsive human papillomavirus-associated lesions. Sex Transm Dis 23:475–480
3. Athie-Morales V, Smits HH, Cantrell DA et al (2004) Sustained IL-12 signaling is required for Th1 development. J Immunol 172:61–69
4. Barratt-Boyes SM, Kao H, Finn OJ (1998) Chimpanzee dendritic cells derived in vitro from blood monocytes and pulsed with antigen elicit specific immune responses in vivo. J Immunolther 21:142–148
5. Bontkes HJ, de Gruijl TD, van den Muysenberg AJ et al (2000) Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. Int J Cancer 88:92–98
6. Boullart AC, Aarntzen EH, Verdijk P et al (2008) Maturation of monocyte-derived dendritic cells with Toll-like receptor 3 and 7/8 ligands combined with prostaglandin E2 results in high interleukin-12 production and cell migration. Cancer Immunol Immunother 57:1589–1597
7. Bowie AG, Haga IR (2005) The role of Toll-like receptors in the host response to viruses. Mol Immunol 42:859–867
8. Buelens C, Verhasselt V, De Groote D et al (1997) Human dendritic cell responses to lipopolysaccharide and CD40 ligation are differentially regulated by interleukin-10. Eur J Immunol 27:1848–1852
9. Cheng WF, Hung CF, Chai CY et al (2001) Tumor-specific immunity and antiangiogenesis generated by a DNA vaccine encoding calreticulin linked to a tumor antigen. J Clin Invest 108:669–678
10. Coccia EM, Severa M, Giacomini E et al (2004) Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. Eur J Immunol 34:796–805
11. Connor JP, Ferrer K, Kane JP et al (1999) Evaluation of Langerhans cells in the cervical epithelium of women with cervical intraepithelial neoplasia. Gynecol Oncol 75:130–135
12. Daling JR, Weiss NS, Hislop TG et al (1987) Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. N Engl J Med 317:973–977
13. Dauer M, Lam V, Arnold H et al (2008) Combined use of toll-like receptor agonists and prostaglandin E (2) in the FastDC model: rapid generation of human monocyte-derived dendritic cells capable of migration and IL-12p70 production. J Immunol Methods 337:97–105
14. De Gruijl TD, Bontkes HJ, Walboomers JMM et al (1998) Differential T helper cell responses to human papillomavirus type 16 E7 related to viral clearance or persistence in patients with cervical neoplasia: a longitudinal study. Cancer Res 58:1700–1706
15. Ferrara A, Nonn M, Sehr P et al (2003) Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. J Cancer Res Clin Oncol 129(9):521–530
16. Frazer IH, Tindle RW, Fernando GJP (1999) Safety and immunogenicity of HPV16 E7/Algammulin. In: Tindle RW et al (eds) Vaccines for human papillomavirus infection and anogenital Disease. RG Landes Company, Austin, pp 91–104
17. Frisch M, Glimelius B, van den Brule AJ et al (1997) Sexually transmitted infection as a cause of anal cancer. N Engl J Med 337:1350–1358
18. Goldstone SE, Palefsky JM, Winnett MT et al (2002) Activity of HspE7, a novel immunotherapy, in patients with anogenital warts. Dis Colon Rectum 45:502–527
19. Haas T, Schmitz F, Heit A et al (2009) Sequence independent interferon-alpha induction by multimerized phosphodiester DNA depends on spatial regulation of Toll-like receptor-9 activation in plasmacytoid dendritic cells. Immunology 126:290–298
20. Haase C, Jorgensen TN, Michelsen BK (2002) Both exogenous and endogenous interleukin-10 affects the maturation of bone-marrow-derived dendritic cells in vitro and strongly influences T-cell priming in vivo. Immunology 107:489–499
21. Holly EA, Whittemore AS, Aston DA et al (1989) Anal cancer incidence: genital warts, anal fissure or fistula, hemorrhoids, and smoking. J Natl Cancer Inst 81:1726–1731
22. Hung CF, Ma B, Monie A et al (2008) Therapeutic human papillomavirus vaccines: current clinical trials and future directions. Expert Opin Biol Ther 8:421–439
23. Lore K, Betts MR, Brenchley JM et al (2003) Toll-like receptor ligands modulate dendritic cells to augment cytomegalovirus- and HIV-1-specific T cell responses. J Immunol 171:4320–4328
24. Matsumoto M, Funami K, Tanabe M et al (2003) Subcellular localization of Toll-like receptor 3 in human dendritic cells. J Immunol 171:3154–3162
25. McKenna K, Beignon AS, Bhardwaj N (2005) Plasmacytoid dendritic cells: linking innate and adaptive immunity. J Virol 79:17–27
26. Melbye M, Cote TR, Kessler L et al (1994) High incidence of anal cancer among AIDS patients. The AIDS/Cancer Working Group. Lancet 343:636–639
27. Memar OM, Arany I, Tyring SK (1995) Skin-associated lymphoid tissue in human immunodeficiency virus-1, human papillomavirus, and herpes simplex virus infections. J Invest Dermatol 105:99S–104S
28. Morel AS, Quaratino S, Douek DC (1997) Split activity of interleukin-10 on antigen capture and antigen presentation by human dendritic cells: definition of a maturative step. Eur J Immunol 27:26–34
29. Mota F, Rayment N, Chong S et al (1999) The antigen-presenting environment in normal and human papillomavirus (HPV)-related premalignant cervical epithelium. Clin Exp Immunol 116:33–40
30. Niedergang F, Didierlaurent A, Kraehenbuhl JP et al (2004) Dendritic cells: the host Achille's heel for mucosal pathogens? Trends Microbiol 12:79–88
31. Renn CN, Sanchez DJ, Ochoa MT et al (2006) TLR activation of Langerhans cell-like dendritic cells triggers an antiviral immune response. J Immunol 177:298–305
32. Santin AD, Bellone S, Gokden M et al (2002) Vaccination with HPV-18 E7-pulsed dendritic cells in a patient with metastatic cervical cancer. N Engl J Med 346:1752–1753
33. Santin AD, Bellone S, Palmieri M et al (2006) HPV16/18 E7-pulsed dendritic cell vaccination in cervical cancer patients with recurrent disease refractory to standard treatment modalities. Gynecol Oncol 100(3):469–478
34. Santini SM, Lapenta C, Santodonato L (2009) IFN-alpha in the generation of dendritic cells for cancer immunotherapy. Handb Exp Pharmacol 188:295–317
35. Schuler-Thurner B, Schultz ES, Berger TG et al (2002) Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. J Exp Med 195:1279–1288
36. Stanley M (2008) HPV vaccines: are they the answer? Br Med Bull 88:59–74

37. Tomai MA, Gibson SC, Imbertson LM et al (1995) Immunomodulating and antiviral activities of the imidazoquinoline S-28463. Antiviral Res 28:253–264
38. van der Burg SH, Ressing ME, Kwappenberg KM et al (2001) Natural T-helper immunity against human papillomavirus type 16 (HPV16) E7-derived peptide epitopes in patients with HPV16-positive cervical lesions: identification of 3 human leukocyte antigen class II-restricted epitopes. Int J Cancer 91:612–618
39. Xu Y, Zhu KJ, Chen XZ et al (2008) Mapping of cytotoxic T lymphocytes epitopes in E7 antigen of human papillomavirus type 11. Arch Dermatol Res 300:235–242
40. Youde SJ, Dunbar PR, Evans EM et al (2000) Use of fluorogenic histocompatibility leukocyte antigen-A*0201/HPV 16 E7 peptide complexes to isolate rare human cytotoxic T-lymphocyte-recognizing endogenous human papillomavirus antigens. Cancer Res 60:365–371
41. Zhao KJ, Cheng H, Zhu KJ et al (2006) Recombined DNA vaccines encoding calreticulin linked to HPV6bE7 enhance immune response and inhibit angiogenic activity in B16 melanoma mouse model expressing HPV 6bE7 antigen. Arch Dermatol Res 198:64–72