Chlamydia trachomatis enhances HPV persistence through immune modulation

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Research Article

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Abstract

Objective: To investigate the immune mechanism of Chlamydia trachomatis (CT) in enhancing the persistent infection of HPV.

Methods: 37 CT/HPV coinfections, 53 HPV infections, 30 CT infections and 30 healthy individuals were enrolled. Antigen-presenting ability, LC density, PI3K and MAPK signaling cascades were detected. Flow cytometry was used to determine the surface activation markers on LC, T cell subgroups and apoptosis.

Results: The antigen presentation ability and LC density in the CT/HPV coinfection were significantly lower than those in HPV and CT infections, and those in the HPV infection group were significantly lower than control ($P<0.05$). The PI3K signaling cascade was further activated, and MAPK pathways were reduced in the CT/HPV coinfection compared with the HPV and CT infection groups. There was less CD4+ T cell, more Treg cell in CT/HPV coinfection than HPV infection group ($P<0.05$); CD8+ T cell becomes fewer but without significance; the CD4+/CD8+ ratio decreased and T cell apoptosis increased in the CT/HPV coinfection group.

Conclusion: CT infection could enhance HPV persistance by inhibiting LC functions, including dysregulating the PI3K and MAPK pathways and reducing LC numbers. CT could also alter T cell subsets and apoptosis, thus impairing cell-mediated immunity and accelerating the progression of cervical cancer.

Introductions

Cervical cancer is the fourth most common malignant tumor among women worldwide[1]. There were approximately 106.2 thousand new cases in China in 2018, ranking first in the world [2], and the disease continues to be a major public health problem affecting the health of women.

Persistent infection with high-risk HPV genotypes (hr-HPVs) is recognized as the most important risk factor in triggering the development of cervical cancer [3].The evidence that *C. trachomatis* may decrease the efficient clearance of HPV infection, favoring HPV persistence, is particularly interesting [4, 5].

HPV and *Chlamydia trachomatis* are the most common sexually transmitted infections globally. According to World Health Organization (WHO) estimates, approximately 290 and 131 million new cases of HPV and *C. trachomatis*, respectively, occur each year[6, 7]. The coinfection rates of HPV and *C. trachomatis* differ in different regions. In southern Hunan Province in China, the incidence is 1.2%[8],while the CT/HPV coinfection rate is 2.7% in Italian females with normal cytology[9].

C. trachomatis has been reported to interfere with viral clearance, supporting the persistence of HPV infection. Two potential mechanisms have been proposed: *C. trachomatis* infection may damage the mucosal barrier and promote the entry of HPV, or it may impair the immune response, favoring the persistence of HPV[5, 10]. A meta-analysis confirmed that patients with CT/HPV coinfection have a higher

risk of cervical cancer[11]. Recently, Abdul Khan reported that *C.trachomatis* coinfection with HPV may modulate cervical cancer development[12].

HPV can achieve persistent infection mainly because it has evolved immune evasion capacity by suppressing the function of Langerhans cells (LCs)[13, 14] and subsequently affects the immune response. LCs are local antigen-presenting cells (APCs)in the epithelium that specialize in the uptake, processing, and presentation of antigens to T cells and induce both CD8+ and CD4+ T cell responses[15]. LCs are responsible for initiating an adaptive immune response against pathogens entering the epithelial layer.

When encountering pathogenic antigens, LCs become activated by phenotypic and functional changes, including the activation of signaling cascades, increased expression of costimulatory molecules and the secretion of inflammatory cytokines. Activated LCs then travel to lymph nodes for cross-presenting HPV peptides to T-cells and initiate an adaptive T cell response against pathogens [16]

However, in HPV infection, LC is less likely to become mature APC but exhibits dysregulation of the PI3K/AKT and MAPK pathways[16]. These two signaling pathways are frequently involved in the regulation of immune responses. After HPV infection, the PI3K signaling cascade is activated, whereas the MAPK pathways are reducedin LC. PI3K signaling cascade activation leads to the upregulation of phosphorylated Akt, and Akt has diverse roles in the regulation of multiple signaling cascades. It can activate NF-κB, downregulate MAPK pathways and inhibit Iκ-B kinase[17], thereby regulating multiple signaling pathways involved in the control of immune responses.

Previous investigations have shown that inhibition of the PI3K cascade can upregulate surface activation markers of LC and induce a potent immune response in HPV infection[18]. HPV-induced immunosuppressive LCs are therefore unable to initiate effective cytotoxic T cell responses against HPV. These data indicate that the activation of the PI3K and MAPK pathways defines a novel immune evasion mechanism of HPV.

Invaded *C.trachomatis* also induces long-lasting activation of the PI3/Akt signaling pathway, which that is required for replication of *C.trachomatis*; however, it is unclear whether MAPK/ERK signaling is regulated in *C. trachomatis* infection[19, 20].

CT interfere with the immune response and allow the persistence of HPV[4, 5]. It has been reported that CT infection can decrease the number of antigen-presenting cells and reduce the antigen-presenting ability of LCs[21]. CT can also affect CD4+ and CD8+ T cells[22]. However, the role of CT in the process of HPV infection has not been fully elucidated.

The primary objective of this study was to investigate whether CT aggravates HPV-induced immune suppression by dysregulating the PI3K/AKT and MAPK pathways and inhibiting the antigen-presenting ability of LCs and whether CT infection can change T cell subsets and induce apoptosis, thus to define

the immune mechanism of CT in enhancing persistent HPV infection and accelerating the progression of cervical cancer.

Results

Expression of antigen presentation and costimulatory molecules on LCs in different groups of patients

LCs expressed low levels of CD40, CD80, CD86 and the maturation marker CD83. The CT infection group had slightly decreased expression of MHC and activation markers compared to the control group. The HPV infection group demonstrated nonsignificant but lower levels of MHC I, MHC II and costimulatory molecules on LCs than the control group, consistent with the idea that HPV is somewhat suppressive of LC activation. In the CT/HPV coinfection group, the expression levels of MHC and costimulatory molecules in the LCs were decreased significantly compared to those in the HPV infection group, suggesting potential immune suppression caused by CT. However, treatment with the PI3K inhibitor LY294002 in the HPV infection group caused significant upregulation of all surface markers analyzed. The results are shown in Fig. 1.

These data show that LCs in the HPV and CT groups did not have notably decreased marker expression, but the CT/HPV coinfection suppressed the level of MHC and costimulatory molecules significantly. However, after treatment with the PI3K inhibitor LY294002in the HPV group, LCs had significantly increased marker expression (Fig. 1). These data indicate that in CT/HPV coinfection, the suppression of PI3K can upregulate the surface markers on LCs

After HPV infection, LCs downregulate MAPK and activate the PI3K pathway

Western blot analysis showed that the phosphorylation levels of PI3K and Akt in HPV infection and CT infection groups were enhanced in LCs compared with the control group, and CT/HPV coinfection elevated the phosphorylation of PI3K and Akt compared with the HPV infection and CT infection groups (Fig. 2a, Fig 2c).

MAPK pathways are suppressed in LCs after HPV infection. LCs showed decreases in p-ERK1/2 and p-MKK4 (Fig. 2b, Fig 2c) compared with the control, and in CT/HPV coinfection, MAPK pathways were much suppressed compared with the HPV and CT infection groups ($p<0.05$) .

Overall, the data indicate that Ct infection affects the signaling pathways involved in HPV infection, and the further activation of PI3K/Akt and the suppression of MARK pathways by CT/HPV coinfection aggravate the suppression of LCs to induce an immune response.

Comparison of the antigen-presenting abilities in different groups of patients

The antigen-presenting abilities of different groups were detected by MLR and compared, and the results are expressed as the percentage of PBMC proliferation (Fig. 3). It was found that the antigen-presenting abilities in both the CT/HPV coinfection and HPV infection groups were significantly decreased compared

to the control group (P<0.05); the CT infection group had decreased antigen-presenting ability compared to the control group, but without significance; and the antigen-presenting ability in the CT/HPV coinfection group was significantly lower than that in the HPV infection group (P<0.05). These results indicate that HPV infection suppressed the function of LCs, and coinfection with CT aggravated the suppression.

Comparison of LC density in different groups of patients

LC density was detected by immunofluorescence staining and presented as CD207. LC density was significantly decreased in the HPV infection group compared with the control group (P<0.01), while LC density in the CT/HPV coinfection group was further decreased, with a significant difference compared with the HPV infection group (P<0.01) (Fig. 4).

Immunofluorescence staining of Tregs

The number of $FOXP3^+$ Tregs increased in HPV and CT groups than control. Comparison of the groups revealed a strong difference in Tregs infiltration between HPV and CT/HPV, CT and CT/HPV (P< 0.05). The CT/HPV group is most strongly infiltrated with Treg cells, as shown in Fig 5.

Flow cytometry of T cell subsets and apoptosis

T cell subsets and apoptosis were detected in each group. In CT/HPV coinfection patients, few or no $CD4^+$ and fewer $CD8^+$ T cells were found, there was significant difference in the frequency of CD4+ T cells between HPV group and CT/HPV group, HPV group and control. Significant difference was detected in the percentage of CD8+ cytotoxic T cells in HPV group compared to control. In addition, there was no significant of CD8+ cytotoxic T cells between the CT/HPV group and HPV group

The apoptosis of T cells in CT/HPV coinfection patients was elevated compared to that of HPV infection patients and controls (Fig. 6).

Discussion

Immune escape and persistence of hr-HPV infection are essential factors for the development of cervical cancer. Generally, most HPV infections are quickly eliminated by the host immune system, and cellular immunity plays a major role in this process[28].

HPV and *Chlamydia trachomatis* are the most common causes of sexually transmitted diseases worldwide. HPV and *Chlamydia trachomatis* share similar risk factors, such as younger age and higher number of sexual partners, CT/HPV coinfection rate increase rapidly in recent years[29].

In coinfection of CT with HPV, the persistent infection of HPV is enhanced[30, 31]. CT/HPV coinfection patients have a higher risk of cervical cancer, CT coinfection with HPV may modulate cervical cancer development[32, 33]. In our research, CT/HPV coinfection patients usually have more serious precancerous

lesions than HPV and CT groups as shown in Table 1. This suggests that CT infection might promote HPV infection and faciliate cervical cancer development.

Chronic CT infection could cause persistent infection of HPV by reducing antigen-presenting cells and inhibiting cell-mediated immunity[30, 31].

A previous study showed that HPV-mediated suppression of LC immune function was a key mechanism by which HPV evades immune surveillance[6, 7]. Human LCs may be immunosuppressive after encountering HPV VLPs, however, they do so in the absence of costimulation[34]. After HPV infection LC activates the PI3K pathway and Akt and modulates both MAPK and NF-κB cascades[35],thereby modulating multiple signaling cascades involved in the regulation of immune responses. PI3K pathway is activated in LCs after HPV infection, CT infection can also activate the PI3K pathway, so CT/HPV coinfection aggravates the activation of the PI3K pathway.

After treatment with a PI3K inhibitor, LCs upregulate surface costimulatory molecules and are capable of initiating an HPV-specific immune response. As the inhibition of PI3K resulted in enhanced immunity[36], CT/HPV coinfection enhanced the activation of the PI3K pathway and downregulated the MAPK pathway, which caused further suppression of immunity. This result indicates that CT causes persistent HPV infection by dysregulating the PI3K/Akt and MAPK pathways in LCs, and the activation of PI3K in LCs defines a mechanism of immune escape used by HPV.

In addition, HPV infection can reduce the LC number and antigen presentation ability compared to that of the control. In HPV infection, the lack of an effective immune response suggests that viral antigens are not adequately presented to T cells. CT/HPV coinfection significantly reduced the LC density and antigen presentation ability compared to HPV infection alone ($P<0.01$). Only when HPV peptides presenting on the LC surface are transferred to T cells, will T cells not respond and may even become suppressed. The decreased LC quantity and antigen presentation ability caused by CT can result in worse local cellular immune function and persistent HPV infection, leading to atypical hyperplasia and cervical cancer.

CT/HPV coinfection also changed the T cell subsets. The results showed few CD^4+ T cells, fewer $CD8^+$ cells, and CD4:CD8 ratio decreased to less than 1. Infiltrated Treg cells were present in the CT/HPV coinfection group. CT/HPV coinfection also aggravated T cell apoptosis.

$CD8^+$ cytotoxic T lymphocyte (CTL)-mediated cytolysis of target cells plays an important role in the process of anti-HPV infection. CD8+ T cells respond to antigens presented in the context of MHC I by LCs following infection. Therefore, the recognition of MHC I by $CD8^+$ cells is a key step in the immune cytotoxicity of $CD8^+$ CTLs. $CD4^+$ T cells can also produce immunity against HPV infection, and CD4+ T cells recognize antigens in the context of MHC II, which are mainly expressed on the surface of LCs in the cervical epithelium. The immune effect may be activated only after MHC II molecules are recognized by $CD4^+$ T cells[38].

Compared with the HPV infection group, CT/HPV coinfection further downregulated the expression of MHC I and MHC II molecules on the LC surface and reduced the $CD4^+$ and $CD8^+$ CTL subsets, impeding T-cell recognition of infected cells, leading to a reduction in CD8+ T cell responses, thus inhibiting cell-mediated immunity and leading to persistent HPV infection.

Regulatory T cells (Tregs) often appear in persistent HPV infection and are positively correlated with the lesion size[39]. The increased level of Tregs in this study was consistent with previous results.

In conclusion, CT infection exacerbates the activation of PI3K/Akt and the suppression of MARK pathways in HPV infection, downregulates surface activation markers and the quantity of LC, impairs the immune cytotoxicity of $CD4^+$ and $CD8^+$ T cells, inhibits cell-mediated immunity and the clearance of HPV, results in enhanced persistent HPV infection, accelerates the natural infection process of HPV and leads to precancerous lesions and cervical cancer.

Moreover, these findings suggest that in addition to a newly identified CT-enhanced immune escape mechanism used by HPV, PI3K may serve as a candidate target for inhibition to enhance HPV immunity.

Material And Methods

Patient material

In this study, 90 patients with HPV 16 infection (the most common hr-HPV type in China) and 30 patients with chronic *C. trachomatis* infection only in Shanghai Zhabei Hospital from July 2017 to July 2018 were enrolled. Written informed consent for blood and tissue sampling was obtained from all individuals under a protocol approved by the Institutional Review Board.

Among 90 patients with HPV 16 infection, 37 cases were in the CT/HPV coinfection group, and 53 cases were in the HPV infection group. Thirty CT infection patients and thirty healthy donors as a control group were enrolled in the study. The patient characteristics are summarized in Table 1. No statistical significance ($P>0.05$) was found between the differences in the general information of each group. Blood samples, cervical exfoliated cells and cervical epithelial biopsy samples (2-5 mm^2) were collected. Exfoliated cells were collected via cervical swab. HPV genotyping was performed using the INNO-LiPA HPV Genotyping Extra kit (Innogenetics, Seguin, TX). CT was screened using the ABON Chlamydia trachomatis antigen detection kit (Fujian,China) and confirmed by Chlamydia TaqMan PCR Kit (Norgen Biotek, Canada).The cytological results were categorized following the 2014 Bethesda System as (a) negative for an intraepithelial lesion or malignant (NILM), (b) atypical squamous cells of undetermined significance (ASCUS), (c) low-grade squamous intraepithelial lesion (LSIL), (d) atypical squamous cells that cannot be ruled out as high-grade squamous intraepithelial lesion (ASC-H), (e) high-grade squamous intraepithelial lesion (HSIL), or (f) squamous cell carcinoma (SCC)[23].

The study was approved by the institutional review board (CWO) of Shanghai Zhabei Central Hospital. All research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants

Table 1. Patient demographics and disease characteristics

Clinical parameter	HPV	CT/HPV	C. trachomatis	control
No. of patients	53	37	30	30
Median age (range)	36.22±6.17(20-65)	35.86±5.42(21-63)	33.43±4.12(18-60)	34.57±7.29(22-60)
Pathological diagnosis				
Negative	39(73.5%)	22(59.4%)	24(80.0%)	
ASC-US	6(11.3%)	3(8.1%)	3(10.0%)	
LSIL	5(9.4%)*	8(21.6%)	2(6.7%)*	
HSIL	3(5.7%)*	4(10.8%)	1(3.3%)*	
Total	53	37	30	

* P<0.05 compared with CT/HPV coinfection group

Langerhans cell generation and costimulatory molecule analysis

LCs from different groups were separated from human PBMCs as previously described[23]. Brie y, PBMCs were incubated in RPMI medium with 1000 U/ml GM-CSF, 2 mM L-glutamine, 10 mg of streptomycin, 10% fetal bovine serum (FBS),1000 U/ml IL-4, and 10 ng/ml TGF-β for 7 days. The LC phenotype was con rmed by ow cytometry as CD1a+Langerin+CD14-.

Surface markers were detected by flow cytometry. Briefly, 10^6 LCs were harvested, washed, stained with MHC I(ab134189,ABCAM), MHC II(ab55152, ABCAM), CD80(ab134120,ABCAM), CD83(ab275021,ABCAM), CD86(ab239075, ABCAM), CD40(ab224639,ABCAM Inc,Cambridge,Uk) Ab or isotype controls, incubated for 1 hr, and washed with FACS buffer. All flow cytometric analyses were performed using an FC500 flow cytometer. Then, we used 20 μM LY294002 (EMD Biosciences), a potent specific inhibitor of PI3K for LC and detected surface markers again. Flow cytometric data were quantified as the median fluorescence intensity (MFI) and analyzed using FlowJo software (FlowJo, LLC).

Immunofluorescence Staining

Density of Langerhans cells (LCs) in cervical biopsy samples were measured. LCs are Langerin (CD207)-positive dendritic cells that constitutively express CD11c[24]. The cervical biopsy samples were cut into frozen sections with a thickness of 5 mm and stained with CD207(ab283686,ABCAM) Ab, followed by

fluorescent conjugated secondary antibody (TRITC). Then, CD11c(ab52632,ABCAM Inc., Cambridge, Uk) were applied, followed by the secondary antibody FITC. Samples were imaged by confocal fluorescence microscopy, and cells visible as fluorescent objects with a size above 4.5 μm were quantified using Image J software (National Institute of Mental Health, Bethesda, USA)[25]

Western blot analysis

LCs from different groups were collected as described above, washed twice with PBS. Cellular extracts were prepared using the Mammalian Protein Extraction Reagent (Pierce). Immunoblotting was performed using Primary antibodies top-ERK1/2(CST,4694S, cell signaling Technology Co.LTD. Massachusett, USA), ERK1/2(CST,4695S), p-MAPK kinase (MKK)4(CST,9151S), MKK4(CST,9152S), PI3K(ab278545), p-PI3K (ab278545,ABCAM Inc.,Cambridge,Uk), AKT(CST,9272S) and p-AKT(CST,13038S) or GAPDH(CST,5174T, cell signaling Technology Co. LTD. Massachusett,USA)

GAPDH was used as internal control as previous reports[26]. Anti-rabbit or anti-mouse HRP-conjugated secondary antibodies were also used. Proteins were visualized using enhanced chemiluminescence Western blotting detection reagents and detected with an HMIAS-2000 Imaging System. Band densities were determined by Bio-Rad Quantity One software and quantified as the ratio to GAPDH.

Antigen-presenting ability analysis

Antigen-presenting ability analysiswas performed using a mixed lymphocyte response assay (MLR)[27]. Cervical epithelial biopsy samples (2-5 mm^2) were incubated with Dispase II (2.4 U/ml) for 45 min and then digested with 0.03% trypsin and 0.02% DNase; approximately (0.05-0.2) $\times 10^6$ epithelial cells could be obtained from each sample. Epithelial cells(including 500-1000 LC) were used as stimulators, and the same amount of PBMCs from the control group was used as a responder. They were mixed and incubated in RPMI 1640 for 3 days.[3H]-TdR (0.5 μCi/well) was added for the final 18 hrs of incubation. Cells were harvested, and incorporation of [3H]-TdR into DNA was measured using a Top Count Microplate Scintillation and Luminescence Counter (Packard Instrument, Meriden, CO). The results are expressed as the proliferation index(mean radioactive cpm (mean counts per minute)/mean cpm).

Flow cytometry

Flow cytometry was used to detect CD4+ and CD8+ subsets as well as the apoptosis of T lymphocytes. PBMCs were separated by using Ficoll density gradient centrifugation. A total of 5×10^6cells/ml from each patient or healthy donor were suspended separately in PBS, stained with monoclonal antibodies CD4-PEcy7 (ab233660, ABCAM Inc.), CD8-APC-Cy7(ab233300,ABCAM Inc.), CD3FITC (ab34275, ABCAM Inc.,Cambridge,Uk), incubated for 1 hr at 4°C and resuspended in cold PBS. All samples were analyzed using a BD FACS Canto II flow cytometry system (BD Biosciences).

The apoptosis of T lymphocytes was assessed using PE-AnnexinV staining (BD Biosciences). Data were analyzed using FlowJo software.

Statistics

SPSS 20.0 statistical software was used to process the statistical data. Statistical significance of the different assays (costimulatory molecule assay, LC density analysis, MLR assay and flow cytometry) was determined by one-way ANOVA for overall significance followed by Tukey's multiple comparisons test. Nonparametric Mann–Whitney U tests were performed for representative experiments of technical replicates of single donors performed in triplicate wells. Results with $P<0.05$ indicated significant differences.

Declarations

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Conflict of interest statement,

No conflict of interests for each author exist

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

References

1. Dora C. Stylianou, Anastasia Constantinidou,and Leondios G. KostrikisCervical Cancer Screening Programs in Europe: The Transition Towards HPV Vaccination and Population-Based HPV Testing Andreas C. Chrysostomou, Viruses. 2018; 10(12): 729–736.
2. Marc Arbyn, Elisabete Weiderpass, Laia Bruni, Silvia de Sanjosé, Mona Saraiya, Jacques Ferlay, and Freddie Bray Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis Lancet Glob Health. 2020; 8(2): e191–e203
3. Wang R, Pan W, Jin L, Huang W, Li Y, Wu D, Gao C, Ma D, Liao S. 2020. Human papillomavirus vaccine against cervical cancer: Opportunity and challenge. Cancer Lett 28;471:88–102.
4. Silins I, Ryd W, Strand A, Wadell G, Törnberg S, Hansson BG, Wang X, Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer. 2005 Aug 10;116(1):110–115

5. Di Pietro M, Filardo S, Porpora MG, Recine N, Latino MA, Sessa R. HPV/ Chlamydia trachomatis co-infection: metagenomic analysis of cervical microbiotain asymptomatic women. New Microbiol. 2018 Jan;41(1):34–41
6. Oakeshott P, Aghaizu A, Reid F, Howell-Jones R, Hay PE, Sadiq ST, Lacey CJ, Beddows S, Soldan Frequency and risk factors for prevalent, incident, and persistent genital carcinogenic human papillomavirus infection in sexually active women: community based cohort study. BMJ. 2012; 22;344:e4168-4175.
7. de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007 Jul;7(7):453–9.
8. Hongliang Chen, Lipei Luo, Yating Wen, Bei He, Hua Ling, Jinwei Shui *Chlamydia trachomatis* and Human Papillomavirus Infection in Women From Southern Hunan Province in China: A Large Observational Study Front Microbiol 2020;11: 827–832.
9. Donatella Panatto, Daniela Amicizia, Silvia Bianchi, Elena Rosanna Frati, Carla Maria ZottiChlamydia trachomatis prevalence and chlamydial/HPV co-infection among HPV-unvaccinated young Italian females with normal cytology Hum Vaccin Immunother 2015;11(1):270–6.
10. Vriend HJ, Bogaards JA, van Bergen JE, Brink AA, van den Broek IV, Hoebe CJ Incidence and persistence of carcinogenic genital human papillomavirus infections in young women with or without Chlamydia trachomatis co-infection. Cancer Med. 2015;4(10):1589–98
11. Zhu H, Shen Z, Luo H, Zhang W, Zhu X (Chlamydia Trachomatis infection- associated risk of cervical cancer: A meta analysis. Medicine (Baltimore) 2016;95:e3077 -3081
12. Abdul Arif Khan, Abdul wahab A Abuderman, Mohd Tashfeen Ashraf, Zakir Khan Protein-protein interactions of HPV- *Chlamydia trachomatis*-human and their potential in cervical cancer 2020 May;15:509–520.
13. Lechien JR, Seminerio I, Descamps G, Mat Q, Mouawad F, Hans S, Julieron M Impact of HPV Infection on the Immune System in Oropharyngeal and Non- Oropharyngeal Squamous Cell Carcinoma: A Systematic Review. Cells. 2019;8(9): 1061–1066
14. Diane M Da Silva, Carly A Movius, Adam B Raff, Heike E Brand, Joseph G Skeate Suppression of Langerhans cell activation is conserved amongst human papillomavirus α and β genotypes, but not a μ genotype Virology 2014;452: 279-86.
15. Banchereau J. and R. M. Steinman. Dendritic cells and the control of immunity Nature 1998; 392: 245–252.
16. Prata TT, Bonin CM, Ferreira AM, Padovani CT, Fernandes CE, Machado AP, Tozetti IA. Local immunosuppression induced by high viral load of human papillomavirus: characterization of cellular phenotypes producing interleukin-10 in cervical neoplastic lesions. Immunology. 2015;146(1):113–121
17. Bossler F, Hoppe-Seyler K, Hoppe-Seyler F.PI3K/AKT/mTOR Signaling Regulates the Virus/Host Cell Crosstalk in HPV-Positive Cervical Cancer Cells. Int J Mol Sci. 2019;20(9):2188–2193

18. Lifang Zhang, Jianhong Wu, Ming Tat Ling The role of the PI3K/Akt/mTOR signaling pathway in human cancers induced by infection with human papilloma viruses Molecular Cancer 2015;14:87–92
19. Prema Subbarayal, Karthika Karunakaran, Ann-Cathrin Winkler, Marion Rother, Erik Gonzalez, Thomas F Meyer, Thomas Rudel EphrinA2 receptor (EphA2) is an invasion and intracellular signaling receptor for Chlamydia trachomatis PLoS Pathog. 2015;11(4):e1004846.
20. Guo Y, Guo R, Zhou Q, Sun C, Zhang X, Liu Y, Liu Q Chlamydiaphage phiCPG1 Capsid Protein Vp1 Inhibits *Chlamydia trachomatis* Growth via the Mitogen-Activated Protein Kinase Pathway. Viruses. 2016;8(4):99–103
21. Diego Del Balzo, Anahí Capmany, Ignacio Cebrian, María Teresa Damiani *Chlamydia trachomatis* Infection Impairs MHC-I Intracellular Trafficking and Antigen Cross-Presentation by Dendritic Cells Front Immunol. 2021;12: 662096-99.
22. Catarina V Nogueira, Xuqing Zhang, Nicholas Giovannone, Erica L Sennott, Michael N StarnbachProtective immunity against Chlamydia trachomatis can engage both CD4+ and CD8+ T cells and bridge the respiratory and genital mucosae J Immunol. 2015;194(5):2319–29.
23. Nayar R, Wilbur DC. The Pap test and Bethesda 2014. Cancer Cytopathol. 2015;123(5):271–81
24. Carrera Silva EA, Nowak W, Tessone L, Olexen CM, Ortiz Wilczyñski JM, Estecho IG, Elena G, Errasti AE, Rosso DA. CD207(+)CD1a(+) cells circulate in pediatric patients with active Langerhans cell histiocytosis. Blood. 2017;130(17): 1898–1902.
25. Biliana Todorova, Nina Salabert, Sabine Tricot Fibered Confocal Fluorescence Microscopy for the Noninvasive Imaging of Langerhans Cells in Macaques Contrast Media & Molecular Imaging 2017, Article ID 3127908, 8 pages
26. Chao Liu, Sayed Sadat, Koji Ebisumoto, Akihiro Sakai, Bharat Panuganti, Shuling Ren Cannabinoids promote progression of HPV positive head and neck squamous cell carcinoma via p38 MAPK activation Clin Cancer Res. 2020; 26(11): 2693–270327.
27. Hermann FJ, Rodriguez Gomez M, Doser K Basophils inhibit proliferation of CD4+ T cells in autologous and allogeneic mixed lymphocyte reactions and limit disease activity in a murine model of graft versus host disease. Immunology. 2015 Jun;145(2):202–12.
28. Harper DM, DeMars LR. HPV vaccines - A review of the first decade. Gynecol Oncol. 2017; 146(1):196–204
29. Marisa Di Pietro, Simone Filardo, Maria Grazia Porpora HPV/*Chlamydia trachomatis* co-infection: metagenomic analysis of cervical microbiota in asymptomatic women New Microbiologica, 2018;41(1): 34–41
30. Marisa Di Pietro, Simone Filardo, Maria Grazia Porpora HPV/Chlamydia trachomatis co-infection: metagenomic analysis of cervical microbiota in asymptomatic women New Microbiol. 2018 Jan;41(1):34–41.
31. Silva J., Cerqueira F., Medeiros R.. Chlamydia trachomatis infection: implications for HPV status and cervical cancer. Arch. Gynecol. Obstet. 2014; 289, 715–723.

32. Haiyan Zhu, Zhaojun Shen, Hui Luo, Wenwen Zhang Chlamydia Trachomatis Infection-Associated Risk of Cervical Cancer:A Meta-Analysis Medicine _ 2016; 95(13)1–10,
33. Human papillomavirus and Chlamydia trachomatis in oral and genital mucosa of women with normal and abnormal cervical cytology J. P. Mosmann, S. Zayas3, A. X. Kiguen, R. F. Venezuela, O. Rosato and C. G. Cuffini BMC Infectious Diseases 2021; 21:422–429
34. Smola S. Human papillomaviruses and skin cancer. Adv Exp Med Biol. 2014; 810: 192–207.
35. Lifang Zhang, Jianhong Wu, Ming Tat Ling, Liang Zhao and Kong-Nan Zhao The role of the PI3K/Akt/mTOR signaling pathway in human cancers induced by infection with human papillomaviruses Molecular Cancer 2015;14:87–92
36. Vielot N, Hudgens MG, Mugo N, Chitwa M, Kimani J, Smith J The Role of Chlamydia trachomatis in High-Risk Human Papillomavirus Persistence Among Female Sex Workers in Nairobi, Kenya Sex Transm Dis. 2015;42(6):305–311
37. Tuong ZK, Lukowski SW, Nguyen QH, Chandra J, Zhou C A model of impaired Langerhans cell maturation associated with HPV induced epithelial hyperplasia. iScience. 2021 Oct 21;24(11):103326
38. Litwin TR, Irvin SR, Chornock RL, Stanley M, Wentzensen N Infiltrating T-cell markers in cervical carcinogenesis: a systematic review and meta-analysis. Br J Cancer. 2021 Feb;124(4):831–841
39. Jung-min Lee, Dana Botesteanui, Yusuke Tomita, Akira Yuno,Min Lee, Elise C. Kohn Patients with BRCA mutated ovarian cancer may have fewer circulating MDSC and more periphera lCD8+ T cells compared with women with BRCA wildtype disease during the early disease course Oncology Letters 2019;18: 3914-3924

Figures

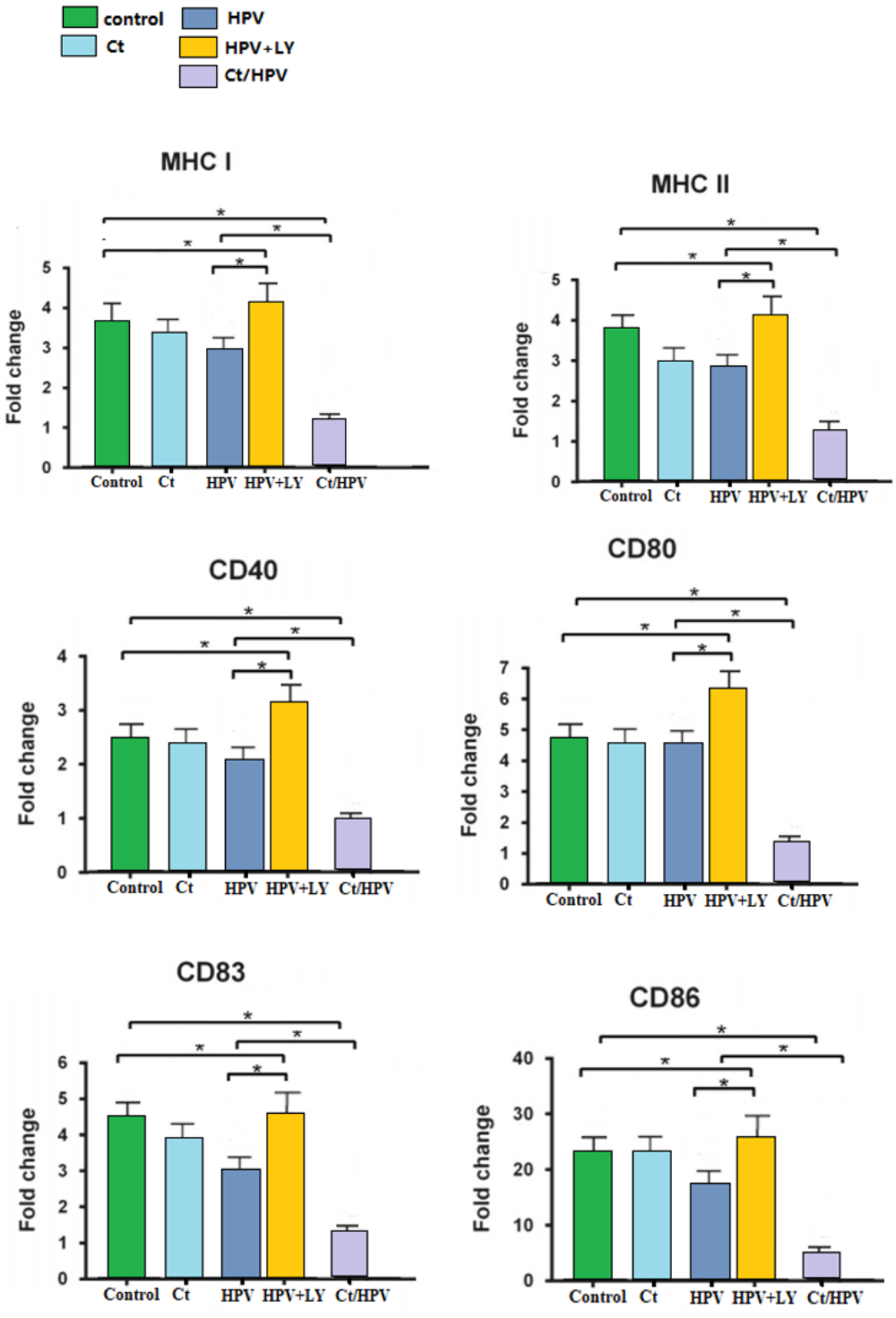
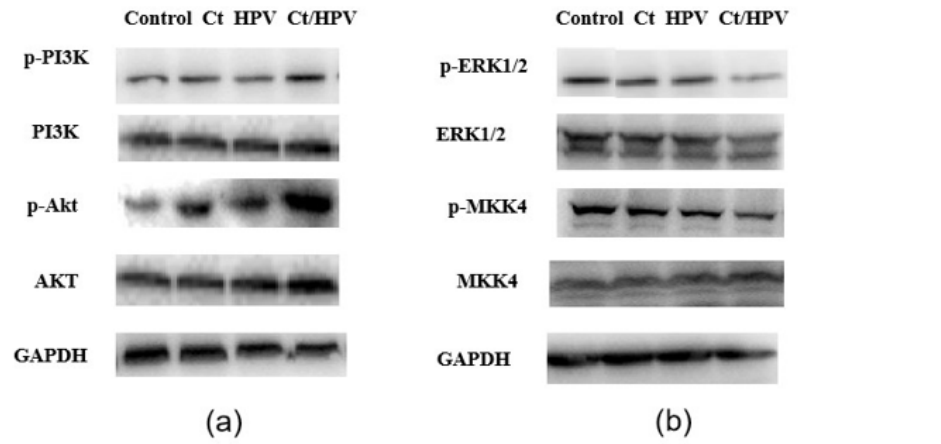


Figure 1

Expression of MHC and costimulatory molecules on LCs in different groups. MHC I, MHC II, CD40, CD80, CD83, and CD86 were assessed via flow cytometry. Data represent the mean fold change in surface marker expression relative to normal LCs based on mean fluorescence intensity (MFI). (N=30 for each group). *p<0.05, compared to the control group (one-way ANOVA, Tukey's posttest).



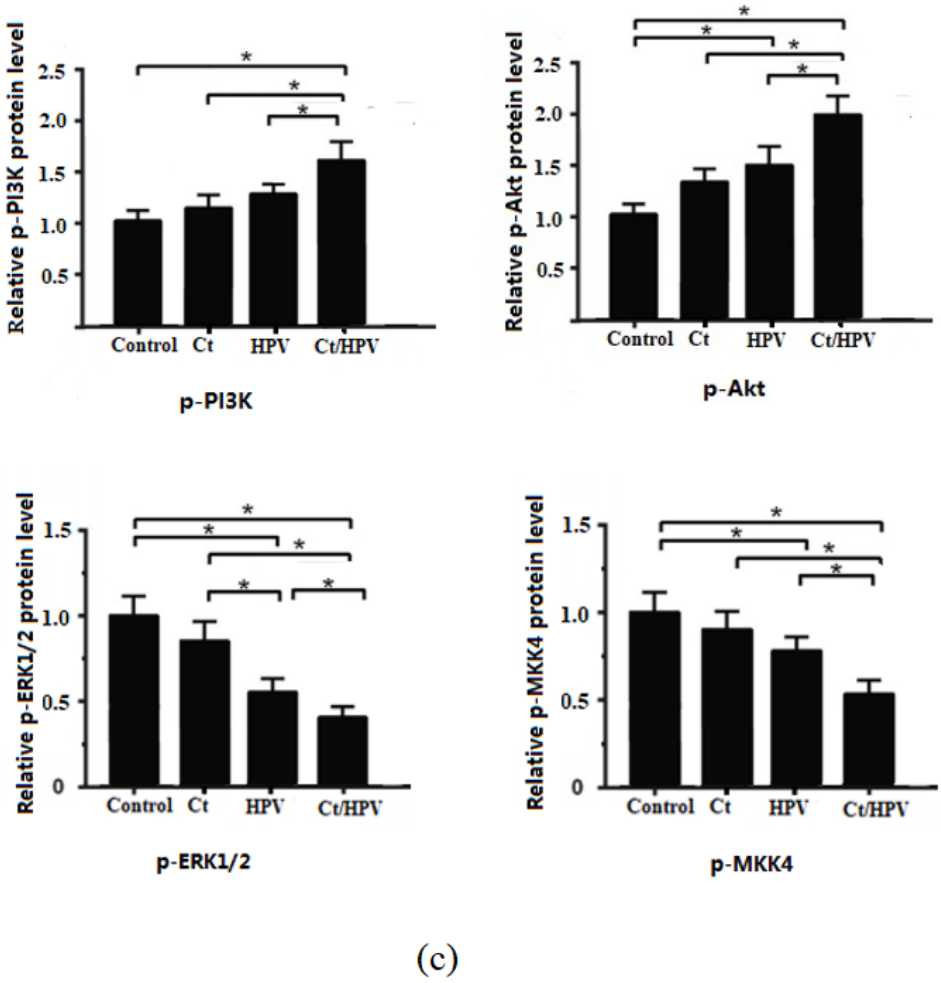


Figure 2

Western blot analysis of the PI3K/Akt and MARK pathways in different groups of patients: (a) activation of PI3K by HPV and CT/HPV coinfection (b) downregulation of the MAPK pathway in HPV and CT/HPV coinfection (c) The expression of MAPK and PI3K pathway proteins (N=30 for each group). *p<0.05, compared to another group (one-way ANOVA, Tukey's test). Gels used in figures are compliant with the digital image and integrity policies.

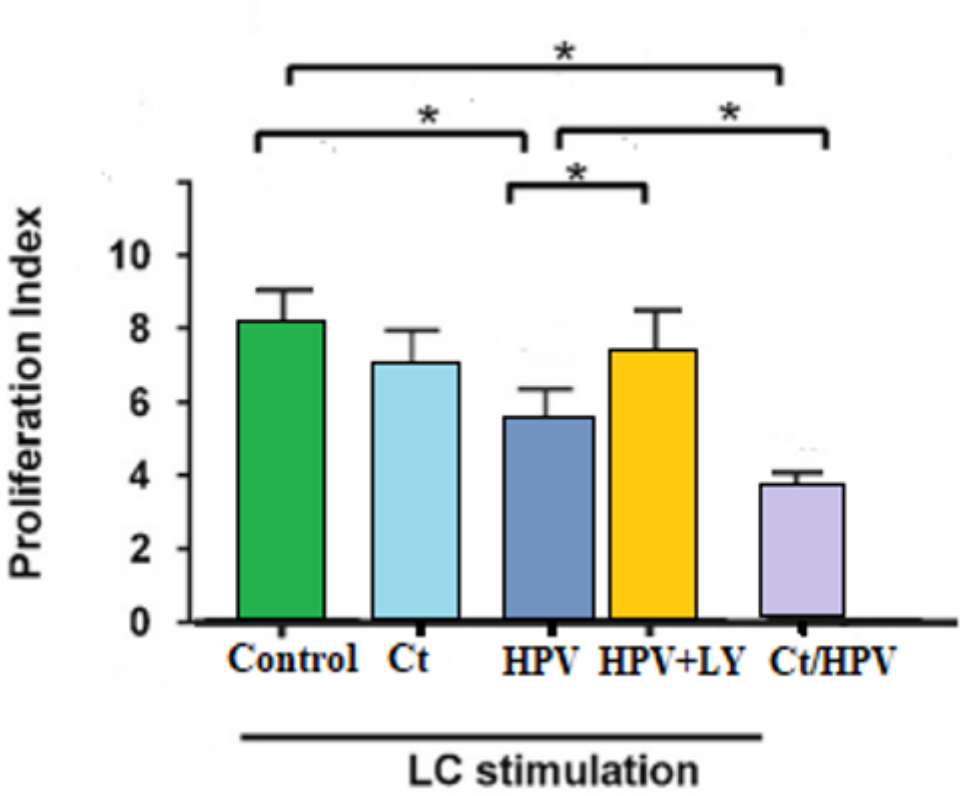


Figure 3

Comparison of the antigen-presenting ability of mixed lymphocytes in different groups (N=30 from each group; differences were determined using one-way ANOVA and Tukey's test for median comparisons. * represent significant differences (P <0.05)

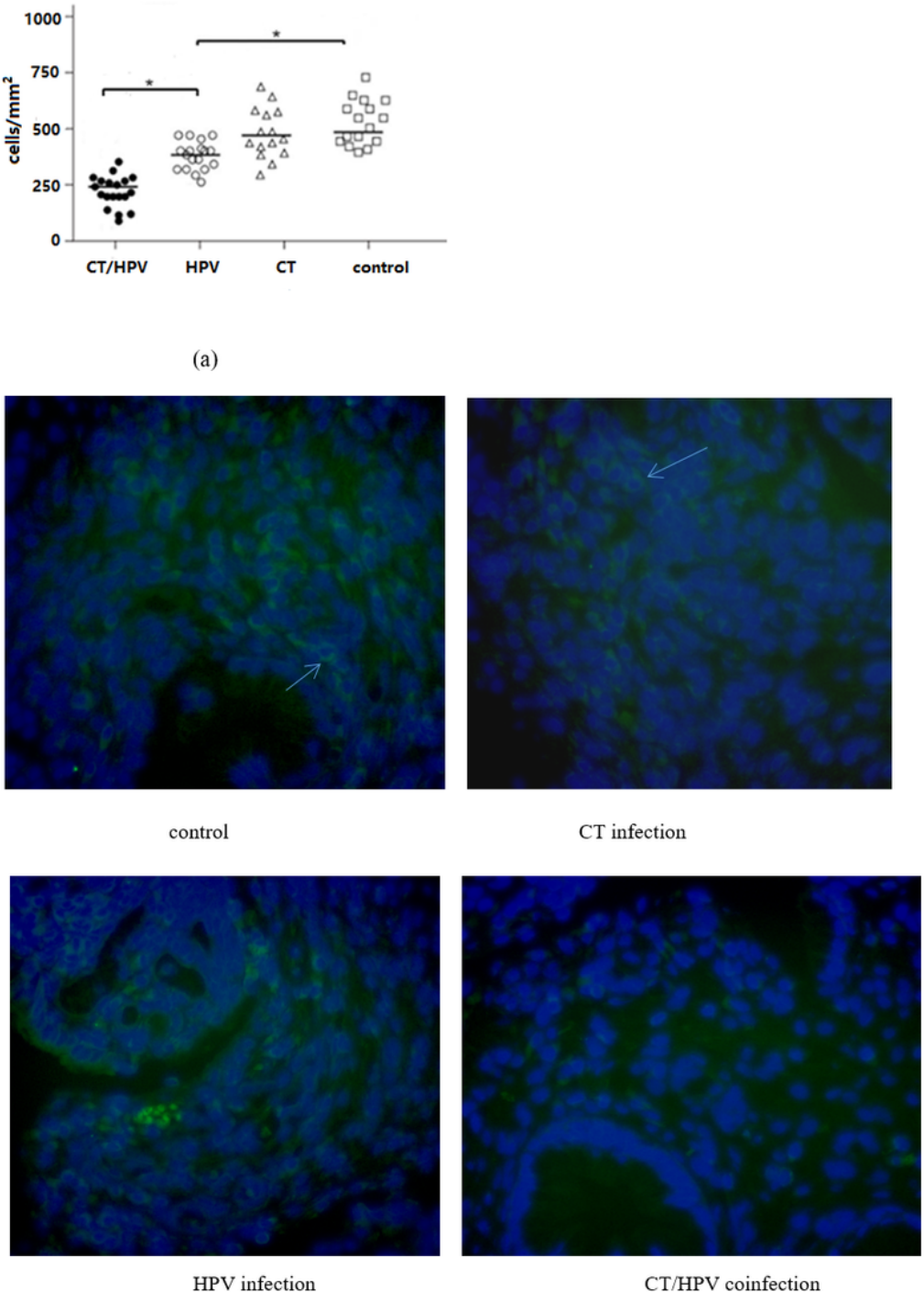


Figure 4

In vivo imaging and quantification of LCs in each specimen group. (a) Quantification of the cell number from different frames that cover an imaging zone of 1 cm in diameter. The results are presented as the mean ± SEM, * represent significant differences (P<0.01) (b) Representative images of Langerhans cells are shown from specimens analyzed in each group. Arrow indicates the CD207-positive cells.

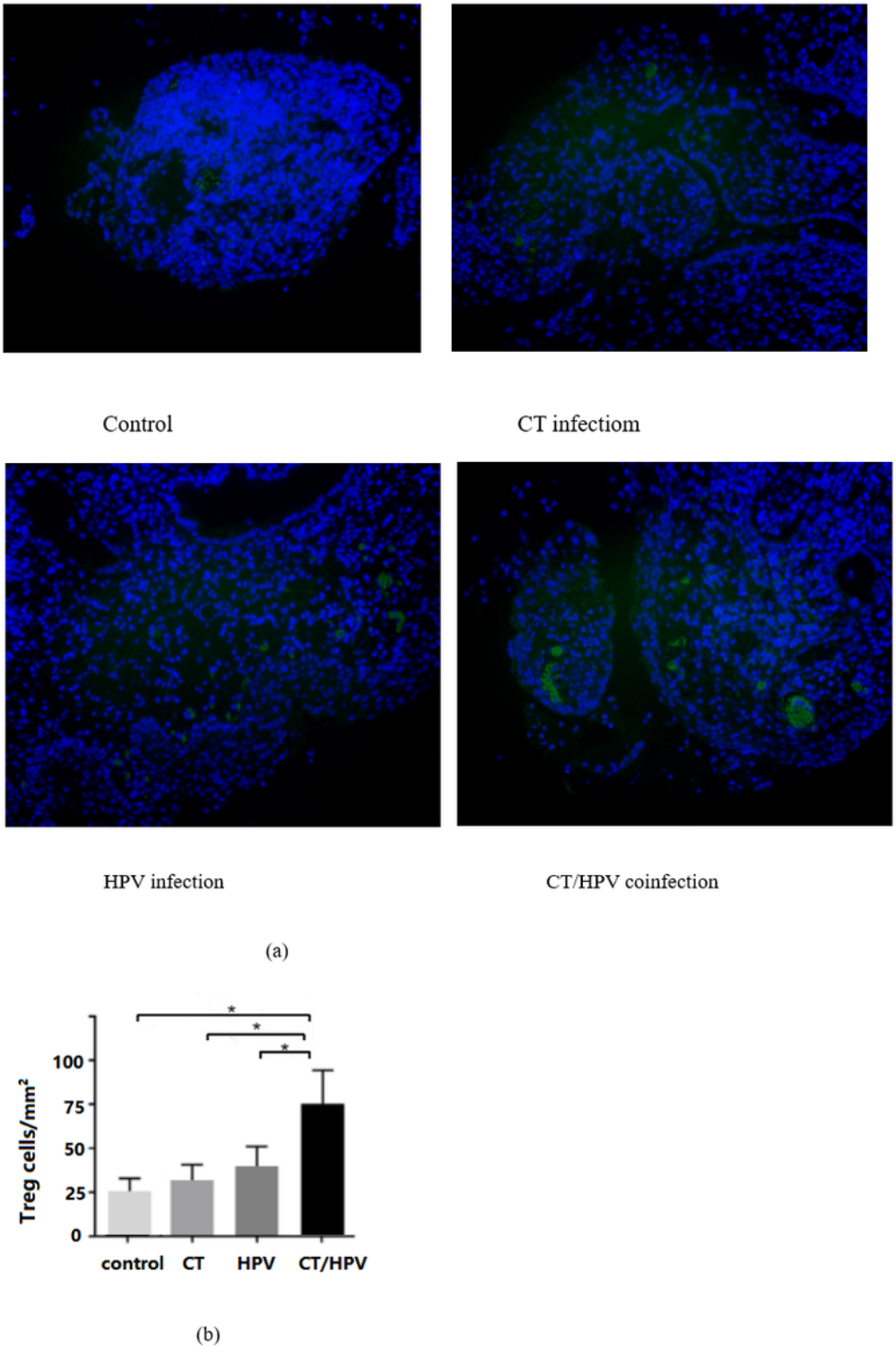


Figure 5

Immunofluorescence staining for FoxP3 (a)Representative image with the combined staining of DAPI in each group (*n* = 30 for each group). Scale bar, 75μm. The arrow indicates FoxP3 positive cells.Green indicates Foxp3^{+},blue indicate DAPI (b) infiltrating regulatory T cells(Tregs) in each group (*n* = 30 for each group). Differences between two groups were calculated with a one-way ANOVA with the significance indicated with asterisks. (*P< 0.05)

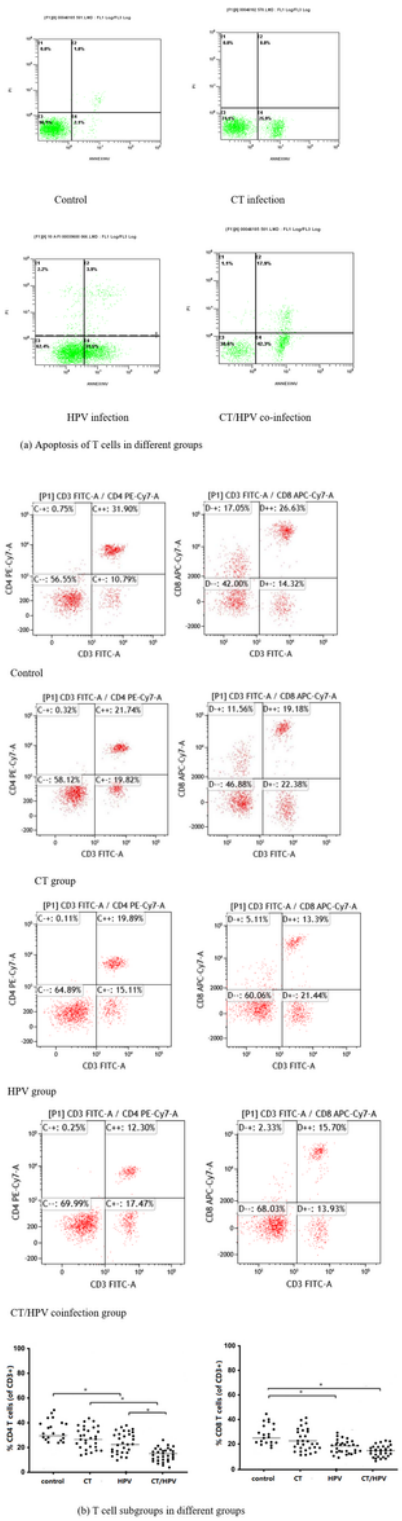


Figure 6

T cell subsets and apoptosis in each group of specimens. (a) Apoptosis of T cells in different groups. (b) T cell subgroups in different groups. Frequency of cervical CD4 and CD8 T cells is shown as percent of CD3+lymphocytes. Each dot represents one patient.

N=30 for each population; differences were determined using one-way ANOVA and Tukey's test for median comparisons. * represents significant differences (P<0.05).