European Review for Medical and Pharmacological Sciences 2017; 21: 1125-1135

5-Aminolevulinic acid photodynamic therapy stimulates local immunity in patients with condylomata acuminata via activation of T lymphocytes

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Abstract. – OBJECTIVE: 5-Aminolevulinic acid photodynamic therapy (5-ALA-PDT) for condylomata acuminate (CA) is effective and safe, but how this treatment affects immune reaction is poorly understood. We aimed to explore the effects of PDT on local immunity in CA.

PATIENTS AND METHODS: Immun[illegible] chemical analysis before and after PDT wa[illegible]er-formed to analyze local changes in the d[illegible]-bution of T lymphocytes, CD123+ plasmacy[illegible] dendritic cells (pDCs) and CD1a+ myeloid DC[illegible] Quantitative Real-time PCR (qP[illegible] was use[illegible] to detect changes in mRNA [illegible]s [illegible]terferon[illegible] (IFN), ISG-15, Mx-2, TLR9, [illegible]RF7.

RESULTS: Compared [illegible] the h[illegible] fore-skin, tissue from pati[illegible] ow[illegible] CD3+ and CD8+ cell[illegible] no[illegible]ant chang-es in CD4+ cells [illegible]D123+ p[illegible] and a sig-nificantly decre[illegible] CD1a+ Lan[illegible]s cells (LCs). Twenty[illegible]s after a P[illegible]ession, local CD3+, C[illegible], an[illegible]23+ pDCs in lesions significan[illegible] increased[illegible] migrated to the superfi[illegible]ermis. CD1a[illegible] in the epider-mis g[illegible]ally decreased, [illegible]le DCs gradu-ally [illegible]eased[illegible]e number, distribution, and mo[illegible]logy. [illegible]D8+ c[illegible]lls did not change af-ter a[illegible]ion. T[illegible]RNA expressions of IFN-γ, I[illegible] IFN-[illegible]G-15, Mx-2, TLR9, and [illegible] were[illegible]ed. As compared to the pa[illegible]ts wit[illegible]gnificantly increased IFN-α and [illegible]N-β after[illegible] PDT session, patients with si[illegible] increases needed fewer sessions [illegible]ure.

CONCLUSIONS: These results suggest that [illegible] for CA can activate T-lymphocyte-meditat-[illegible]munity, and pDC-related immunity is al-so [illegible]tivated. The clinical efficacy of 5-ALA-PDT against CA may be related to the increased IFN-α and IFN-β after treatment.

Words:
[illegible]ondylomata [illegible]minata, Plasmacytoid dendritic [illegible] Myeloid den[illegible]c cells, HPV, Toll-like receptors

Introduction

[illegible]ylomata acuminata are a common sexually transmitted diseases closely related to HPV infection and the body's immune status. Worldwide, ~80% of individuals under the age of 50 have been infected with HPV[1], but not all of those infected with HPV will develop condylomata acuminata. Low activity of local host immune cells[2] may contribute to the development of the disease. Wart removal is the traditional primary treatment, but it cannot prevent recurrence effectively. In 1996, 5-aminolevulinic acid photodynamic therapy (5-ALA PDT/PDT) was first used to treat patients with condylomata acuminata, and it is still widely used almost 20 years later. Several domestic and international studies have shown that PDT results in satisfactory clinical outcomes whether it is applied alone or in conjunction with laser, cryotherapy, or other conventional treatments. PDT is not only clinically effective, but is highly safe and can prevent recurrence[3-6]. Current research suggests that significant immune reactions occur in the course of PDT for condylomata acuminata, with CD4+ T lymphocytes and dendritic cells most likely mediating these reactions[7]. Whether other immune cells also participate in these immune reactions has not been determined. T lympho-

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cytes are the primary mediators of cell-mediated immunity. The number of $CD3^+$ T lymphocytes can represent the total number of T lymphocytes. The primary function of $CD4^+$ T lymphocytes is the secretion of the cytokine interferon (IFN) γ, which strengthens the immune response and activates cell-mediated immunity. Some $CD8^+$ T lymphocytes have immunosuppressive activity, and primarily inhibit immune processes.

Dendritic cells include plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs). pDCs are a special type of dendritic cells that express the surface markers CD123 and BDCA-2[8] and perform an essential function in antiviral immunity; they are also the primary cells that produce type I IFN[9,10]. Type I IFN primarily includes IFN-α and IFN-β. IFN production relies on the MyD88 pathway. After recognizing and binding to viral dsDNA, TLR9 associates with MyD88, resulting in IRF7 activation and production of IFN-α and IFN-β. Patients with condylomata acuminata exhibit local inhibition of immunity. HPV infection further downregulates type I IFN[11-13], causing local immune suppression and inhibited immune status in condyl... acuminata. ISG-15, a key effector protei... type I IFN pathway[14], is known to promote ...ral activity. The Mx protein, an antiviral pr... whose production is induced by type I IFN, ... capture viruses at early stages ...vent vi... replication[15,16].

mDCs, commonly kno... as den...ic cells (DCs), express the sur... marke... and are specific antigen-... they are also imm... cells ... skin. DCs in the epidermis ...own as La...hans cells (LCs), can re... take up, ...present antigens an... immune reactions. After HPV ...fection, L...e-up an HPV antigen an...en present it i... draining lymph node ...ivatin... T lymphocytes there. After acti...on, T ...phocytes migrate to the site of i...n and diss... HPV-infected keratinocyt... wever... immune function is ... wea... i...atients with condylomata ac...ata.

I... he presen... study, we used immunohisto... evaluate local changes in the ...ribution of various types of immune cells ... condylomata acuminata before PDT and 4 ...24 h after a PDT session: $CD3^+$, $CD4^+$, and ...$D8^+$ T lymphocytes; $CD123^+$ pDCs; and $CD1a^+$ DCs. Real-time quantitative PCR (qRT-PCR) was used to evaluate changes in mRNA expression levels of the local-immunity-related factors IFN-α, IFN-β1, IFN-γ, ISG-15, Mx-2, TLR9, and IRF7 in lesions before PDT and 4 and 24 h after a PDT session. Anal... effects of PDT on local immune f...ion a... further assessment of the relati...etween lo... cal immunity and the clinical e...y of PDT can elucidate the immunol...gical ...nisms of PDT.

Patients ... Me...ods

Patients

A total of ...atients, inc... ...males and 11 females ... first onset ...condylomata acuminat... who w... ...ated in the Dermatology Clinic of the Chine... ...ople's Liberation Army ... Hospital be... January 2013 and D...mber 2014 were selected (Table I). All the ...ents signed ...ormed consent forms. Inclu... criteria con...sed the diagnostic criteria of a...yloma ac...natum, positive results of the ace... diagnosis confirmed by histopatho... examination; 18-60 years of age; first onset of a rash without any prior treatments ...ag local and systemic medications and ...ys... therapy); no other sexually transmitted diseases; and no other systemic or autoimmune diseases. Exclusion criteria included patients under 18 or over 60 years of age; rashes consisting of fewer than 8 individual warts; a rash that did not have the first onset, or patients who received prior treatments; the presence of another sexually transmitted disease; and the presence of a systemic or autoimmune disease. The study was approved by the Institutional Review Board of Huashan Hospital, and was conducted in accordance with the Declaration of Helsinki.

PDT

The enrolled patients were tested for the HPV genotype using a routine procedure, after which PDT was initiated. Local lesions were treated with 10% ALA gel (topical 5-aminolevulinic acid HCl topical gel; Shanghai Fudan-Zhangjiang Biopharmaceutical Co., Ltd., Shanghai, China) packed in a plastic wrap and a lightproof plastic wrap. After 3 h, 635-nm red light with 80-120 J/cm^2 energy density (wavelength 635 ± 5 nm, power 0-300 mW, adjustable) was applied. Neither local nor systemic immunomodulators were administered either before or after PDT sessions.

Table I. Characteristics of selected patients.

Patient ID	Sex	Age (y)	Infection duration (months)	Location of lesions	Number of lesions	HPV genot
1	F	25	4	Vulva	10	
2	M	22	2	Perianal area	16	, 11, 58
3	F	24	3	Vulva, perianal area	35	11
4	F	26	4	Vulva	14	18
5	F	60	6	Perianal area	29	
6	F	45	8	Vulva, perianal area	4	6,
7	M	48	10	Penis		6, 11
8	M	49	3	Perianal area		6, 11, 58
9	F	43	2	Vulva	3	6, 11
10	M	27	5	Penis		6,
11	F	35	3	Perianal area		6
12	F	22	3	Vulva, perianal	25	, 58, 68
13	M	26	2	Penis	10	,11
14	F	31	1	Vulva, peri	18	6,11
15	M	48	5	Perianal	24	6,11,18
16	M	39	4	Perianal area	33	6, 11
17	M	50	7	Peni al area		6, 11
18	M	55	2	a		6, 11, 58
19	F	26	1	a	16	6, 11
20	F	23	6	va, perianal are	31	6, 11

F: female; M: male

Immunohistochemical Staining

With the informed consent of patients × 5 × 5 mm biopsies of lesion tissues (inc g wart tissue and superficial dermis) were co ed before a PDT session and 4 and 24 h afte PDT session. One biopsy samp placed 5 ml of 4% paraformaldehyd ight fix- ation at 4ºC. The followin y, tiss samples were placed in 70% eth for sto om temperature, followed y d n p wax. Healthy fores issue es from circumcisions colle n the Urol epartment of the People's L Army Gen Hospital served as h y co and were processed in the sam vay as the tissues were. For immunoh chemical anal mouse anti-human m oclonal ntibodies (Beijing Zhongshan Jin Co., L Beijing, China) against CD3 (1:5 DA 00), C (1:500), CD123 (1:50), and CD 500) w used. The other biopmple mi with the TRIzol Reagent (In gen, d, CA, USA) and stored at -80 Total RN was collected from the tissue, r bed into cDNA, and amplified by -PCR using custom-designed primers (Table II). The mRNA expression levels of IFN-γ, IFN-β, ISG-15, Mx-2, TLR9, and IRF7 we measured before and after treatment. According to the quality of RNA collected, 8 patients were selected for qRT-PCR analysis.

Immu ochemical samples were examined under a light microscope using the dou- d method. Each slide was examined at w ( ) and high (40×) magnification. Five visual fields containing a concentrated distribution of positively stained cells were selected, and the distribution and morphological changes of these cells were recorded. For measurement of mRNA expression levels of immunity-related factors by qRT-PCR, expression levels at 0 h

Table II. Sequences of primers used for quantitative Real-time PCR.

Primers	Sequence (5′ → 3′)
IFN-α	F: GCTTGGGATGAGACCCTCCTA
	R: CCCACCCCCTGTATCACAC
IFN-β	F: ATGACCAACAAGTGTCTCCCTCC
	R: GGAATCCAAGCAAGTTGTAGCTC
IFN-γ	F: GGCTTTTCAGCTCTGCATCG
	R: TCTGTCACTCTCCTCTTTCCA
ISG15	F: CGCAGATCACCCAGAAGATCG
	R: TTCGTCGCATTTGTCCACCA
Mx2	F: CAGAGGCAGCGGAATCGTAA
	R: TGAAGCTCTAGCTCGGTGTTC
TLR-9	F: AATCCCTCATATCCCTGTCCC
	R: GTTGCCGTCCATGAATAGGAAG
IRF7	F: CCCAGCAGGTAGCATTCCC
	R: GCAGCAGTTCCTCCGTGTAG
GADPH	F: CAATGCCAGCCCCAGCGTCA
	R: CAATGCCAGCCCCAGCGTCA

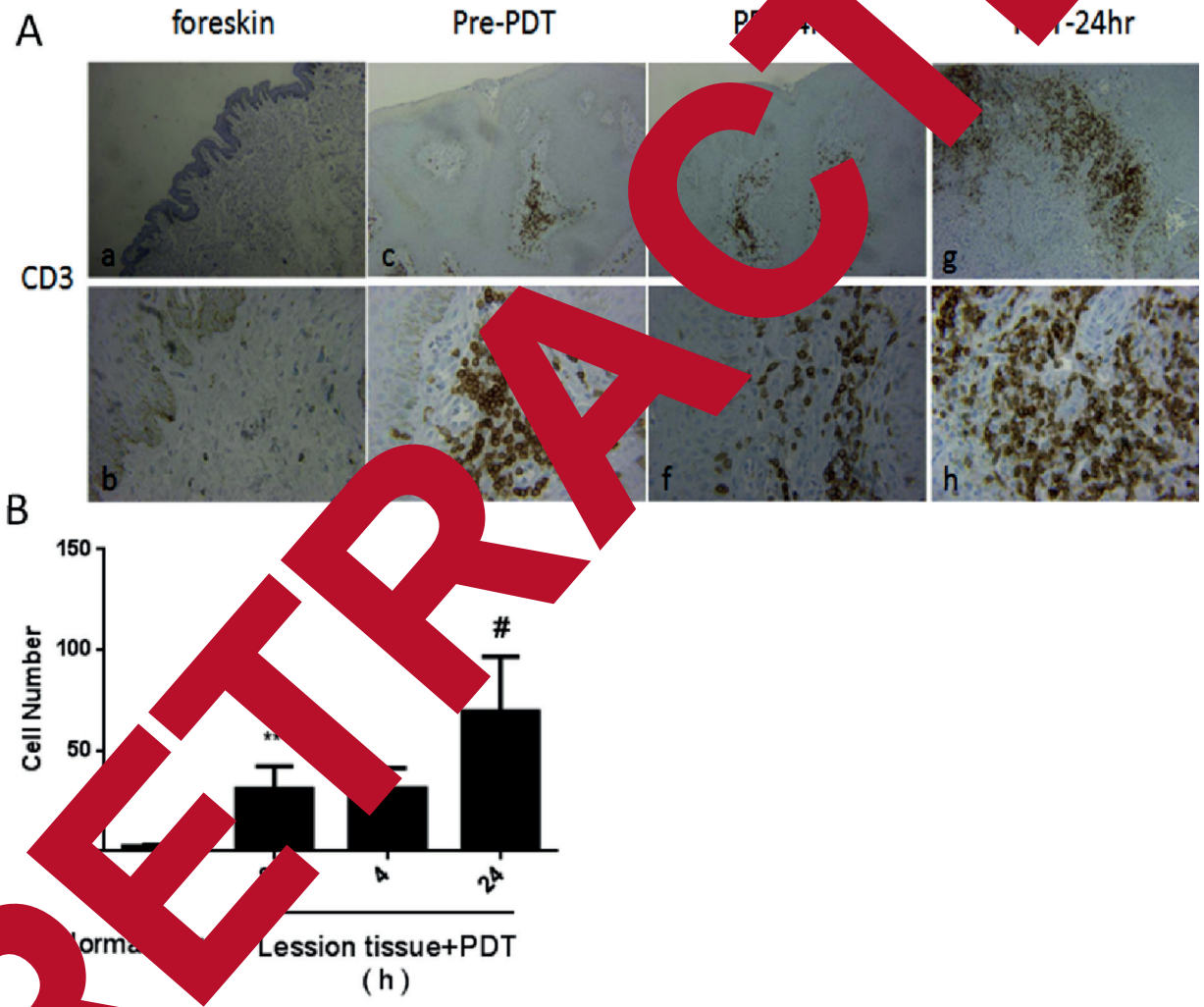
served as a reference, and expression levels at 4 and 24 h were compared to the reference levels, and a fold difference was calculated.

Statistical Analysis

SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis of the differences. Significant effects between treatment and control groups were analyzed using the Student's t-test. Statistical significance was considered when the p value was less than 0.05.

Results

As compared to the healthy foreskin samples, the $CD3^+$ cell number was el... condylomata acuminata samples ...d the... cells were primarily distribut... ...n the superficial dermis. At 4 h after ... ...T session, $CD3^+$ cells showed no sig...ifican... ...nge in the number or distributi... At 24 ... ... a PDT session, the numb... $CD3^+$ cells s... cantly increased, and ...se cell... ...adually ...



...ohistochemical staining for CD3. ***A.*** The small number of $CD3^+$ cells in the superficial dermis of ...thy foresk... tissue. **(a)** 10× and **(b)** 40× magnification. A medium number of $CD3^+$ cells is observed in the middle ...erficial dermis of a condylomata acuminata sample before a PDT session. **(c)** 10× and **(d)** 40× magnification. At 4 h ... PDT session, a medium number of $CD3^+$ cells is observed in the middle-superficial layer of the dermis. **(e)** 10× a... 0× magnification. At 24 h after a PDT session, a large number of $CD3^+$ cells is observed in the superficial dermis, and ... cell distribution is suggestive of migration. **(g)** 10× and **(h)** 40× magnification. B. The cell numbers (mean ± SD) were determined by immunohistochemical staining; $^*p < 0.05$ compared with healthy tissue, $^{\#}p < 0.05$ compared with untreated lesion tissue.

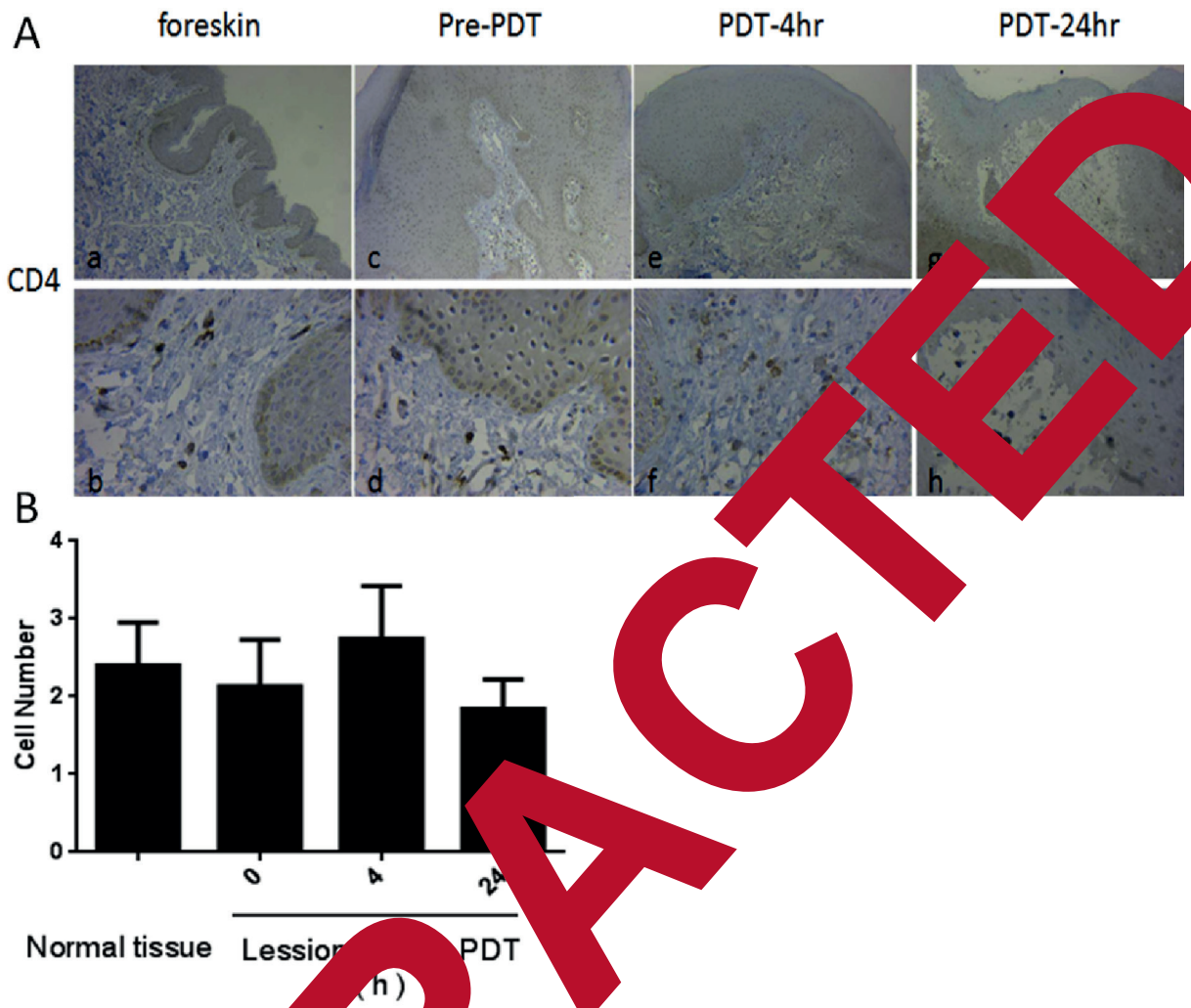


Figure 2. Immunohistochem[illegible]ining [illegible]ow magnification (10×), a small number of CD4⁺ cells can be observed in the superfici[illegible]derm[illegible]y fores[illegible]issues. **(a)** 10× and **(b)** 40× magnification. Few CD4⁺ cells can be detected in the midd[illegible]perfici[illegible]of the dermis of condylomata acuminata samples before PDT. **(c)** 10× and **(d)** 40× magnification. A[illegible] after a PD[illegible]on, few CD4⁺ cells can be observed in the superficial dermis. **(e)** 10× and **(f)** 40× magnificati[illegible]4 h after PDT, [illegible]D4⁺ cells can be observed in the superficial dermis. **(g)** 10× and **(h)** 40× magnification.

grate[illegible]m the[illegible]iddle super[illegible]icial part of the der[illegible]o the[illegible]erficial dermis (Figure 1). As com[illegible]t[illegible]e heal[illegible]foreskin, CD4⁺ cells in cond[illegible]ta acu[illegible]ata samples showed no [illegible]fican[illegible]n number, distribution, or [illegible]phol[illegible]4⁺ cell numbers increased 4 h[illegible]er a PDT session ($p < 0.01$; Figure 2). I[illegible]with healthy foreskin control, [illegible]8⁺ cell [illegible]mbers increased in condylomata [illegible]minata samples ($p < 0.05$), whereas their [illegible]bution and morphology were not signifi-ca[illegible]different. At 4 and 24 h after a PDT session, no significant differences in CD8⁺ cell number, distribution, or morphology were observed (Figure 3). As compared to the healthy foreskin control, CD123⁺ pDCs in condylomata acuminata tissue showed no significant change in their number ($p > 0.05$). At 4 and 24 h after a PDT session, the number of CD123⁺ pDCs in lesion tissue showed an increasing trend ($p < 0.01$; Figure 4). In comparison with the healthy foreskin control, the number of CD1a⁺ LCs in the epidermis of condylomata acuminata tissue was significantly decreased ($p < 0.01$), and the protrusions of the DCs were shorter and fewer. After PDT treatment, CD1a⁺ LCs in the epidermis of condylomata acuminata gradually decreased in number (p

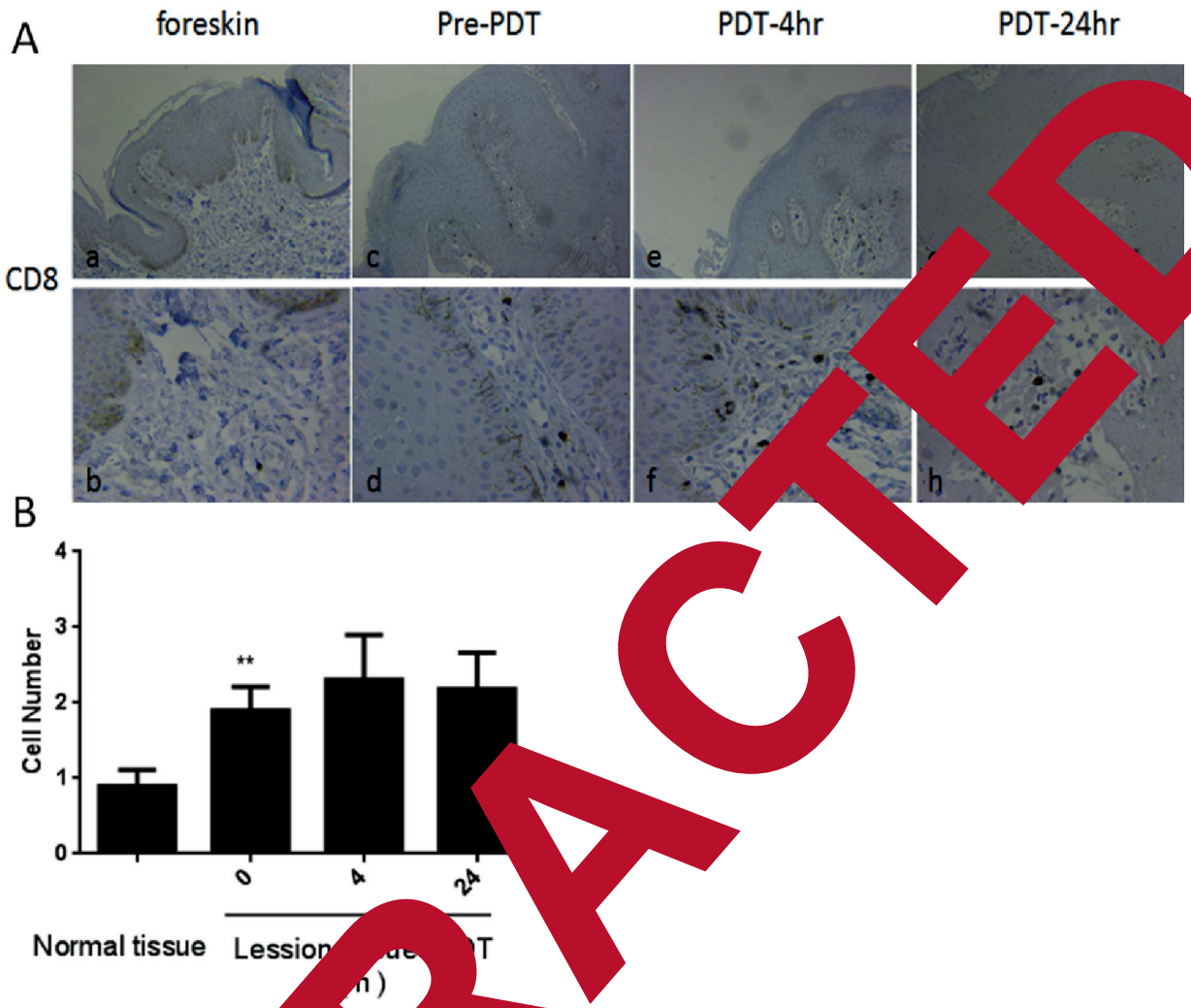


Figure 3. CD8 immunohi toch l st g number of CD8⁺ cells can be observed in the superficial dermis of healthy foreskin tissu) 10 40× magnification. Few CD8⁺ cells can be observed in the middle superficial layer of dermis of co mata acu amples before PDT treatment. **(c)** 10× and **(d)** 40× magnification. **(c)** At 4 h after a PDT sessi high magnific 40×), few CD8⁺ cells can be observed in the superficial dermis. **(e)** 10× and **(f)** 40× magn 24 h after PD w CD8⁺ cells can be observed in the superficial dermis. **(g)** 10× and **(h)** 40× magnificat *B,* Th numbers (mean ± SD) were determined by immunohistochemical staining; $^{**}p < 0.01$ compared wi healthy tissue

< 0. numb of DCs in the dermis gradua reased ese changes could be th migration of LCs located an epide prickle cells or basal cells to t dermis (F gure 5).

alysis of IFN-γ mRNA in lesion ues before a PDT session and 4 and 24 h after atment revealed a significant increase in the sion level at 4 h after treatment ($p < 0.05$) and turn to the original expression level 24 h after treatment (Figure 6). At 4 and 24 h after treatment, the mRNA expression levels of IFN-α, IFN-β, ISG-15, Mx-2, TLR9, and IRF7 significantly increased in local lesion tissues ($p < 0.05$; Figure 7).

In the present study, all 20 patients were cured by PDT. We analyzed 8 patients in whom the clinical efficacy correlated with IFN-α and IFN-β mRNA levels. Five patients exhibited a more than twofold increase in IFN-α and IFN-β levels, and the average level corresponding to cure was 4 ± 0.71. Three patients showed a less than twofold increase or decrease in IFN-α and IFN-β levels, and the average level corresponding to cure was

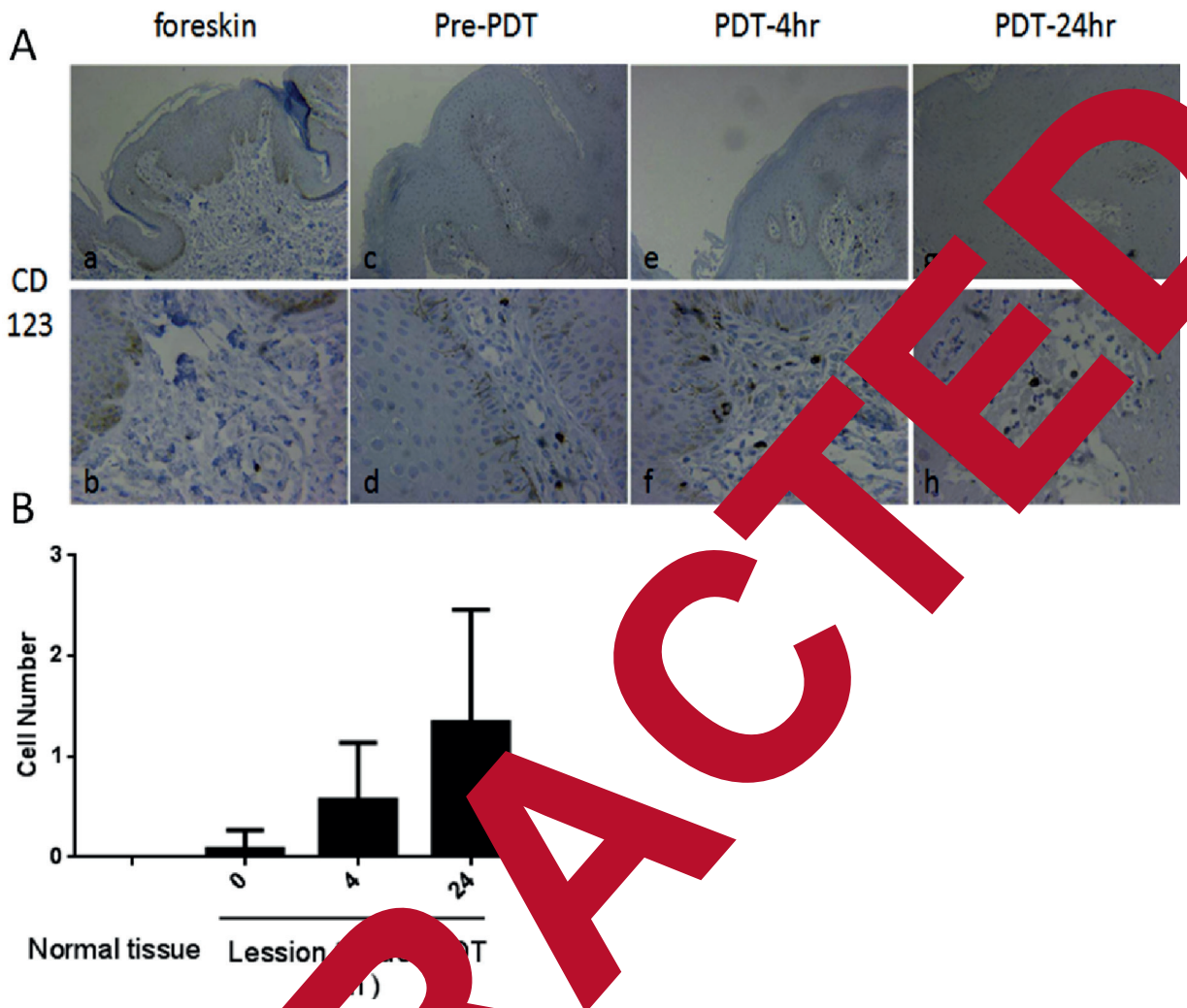


Figure 4. Immunohistochem… ining… s. No CD123⁺ cells can be observed in the epidermis or dermis of healthy foreskin tissu… **(a)** … 40× mag…fication. No CD123⁺ cells can be observed in the epidermis of condylomata acuminat… ples be… T; small numbers of individual CD123⁺ cells can be detected in the superficial dermis. **(c)** 10× and … 0× magnific… At 4 h after a PDT session, CD123⁺ cells are distributed in the superficial dermis. **(e)** 10× ar… magnificatio… 4 h after PDT treatment, CD123⁺ cells are distributed in the superficial dermis. **(g)** 10× … **h)** …gnification.

6 ± 1… Amo… the patient… with significant inc… s in IF… and IFN-β levels (more than two… cr…), the l… needed for a cure was lower t… t in th… tients without a signif- … incre… a… ease or less than twofold inc… e). Th… ence was statistically signif- ica… $p < 0.05$; Table III).

Discussion

Because the patients enrolled in this study had relatively more lesions and had a relatively more serious medical condition, the number of PDT sessions was greater than in other reports[3,8]. Nevertheless, our results still confirm the efficacy and safety of PDT. In the present study, we used biopsies, immunohistochemistry, qRT-PCR, and monitoring of dynamic changes in immune cells and the related factors before and after a PDT session. We used healthy foreskin tissue as a control.

This study revealed that in comparison with healthy foreskin tissues, condylomata acuminata contained increased numbers of CD3⁺ cells, which were primarily distributed in the superficial dermis and had abundant cytoplasm. No significant differences in number, distribution, or morphology of CD4⁺ cells were found, and CD8⁺

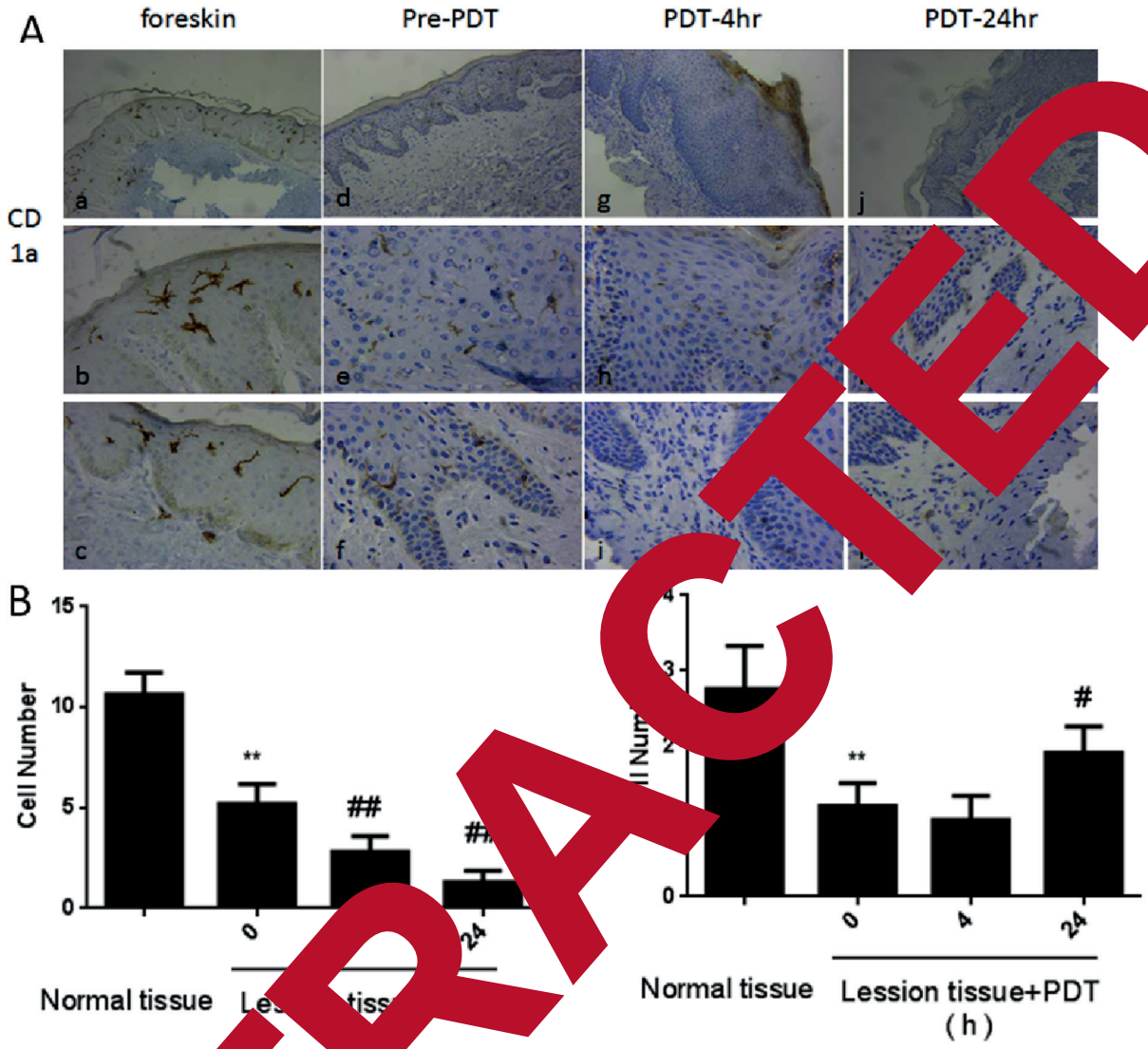


Figure 5. Immuno[illegible]hemical stai[illegible] CD1a$^+$ pDCs. In healthy foreskin tissues, CD1a$^+$ cells are primarily distributed in the [illegible] with few bei[illegible]sent in the dermis. **(a)** 10× and **(c)** 40× magnification. Their cytoplasm and cell membr[illegible]are s[illegible]brown, and th[illegible]r cell body shape is irregular, showing many dendritic protrusions. **(b)** 40× magnificatio[illegible] In condyloni[illegible]minata tissue before PDT, CD1a$^+$ cells are primarily distributed in the epidermis, with few being [illegible]t in the dermis[illegible]0× and **(f)** 40× magnification. CD1a$^+$ cells in the epidermis are primarily distributed among e[illegible]mal prickle cells or [illegible]cells. Their cytoplasm and cell membranes are stained brown, and in comparison with h[illegible]y fores[illegible]tissues, the cellular protrusions are shorter and fewer here. **(e)** 40× magnification. At 4 h after a PDT[illegible]on, CD[illegible]ells are primarily distributed among epidermal prickle cells or basal cells, with few being observed in th[illegible]is [illegible]0× and [illegible]40× magnification. Dendritic protrusions are shorter and fewer. **(h)** 40× magnification. At 24 h af[illegible]session [illegible]a$^+$ cells can rarely be observed in the epidermis but can be found in the dermis. **(j)** 10× and [illegible]40× ma[illegible]ation [illegible]a$^+$ cells in the dermis are spindle-like or dot-like. **(h)** 40× magnification. ***B,*** The cell numbers [illegible]SD) [illegible]ined by immunohistochemical staining; $^{**}p < 0.05$ compared with healthy tissue; $^{\#}p < 0.05$, $^{\#\#}p < 0.$[illegible]ompared [illegible]ntreated lesion tissue.

[illegible]s increased in number and were primarily [illegible]tributed in the superficial dermis of lesions. [illegible] data confirm local immune suppression in [illegible]ndylomata acuminata tissue and inhibition of antiviral immunity. Comparison of condylomata acuminata samples before and after a PDT session revealed that the CD3$^+$ cell number did not change significantly 4 h after treatment, but increased significantly at 24 h. The CD4$^+$ cell number increased at 4 h after treatment, and CD4$^+$ cells migrated upward from the middle superficial layer of the dermis to the superficial

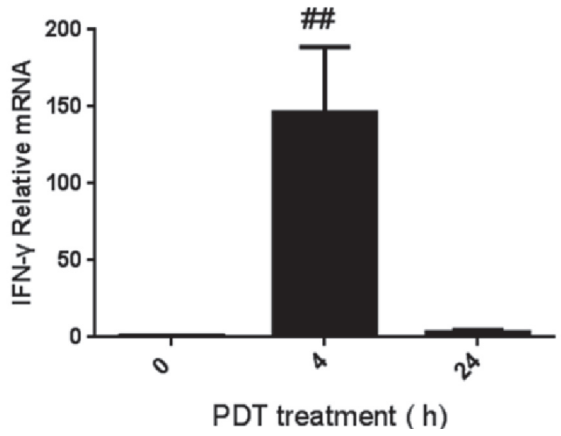


Figure 6. Relative expression levels of IFN-γ in lesions before PDT and 4 and 24 h after a PDT session. $^{\#\#}p < 0.01$ compared with untreated lesion tissue.

dermis. qRT-PCR analysis revealed that condylomata acuminata tissue expressed increased levels of IFN-γ mRNA 4 h after a PDT session. Currently, it is widely believed that IFN-γ is a cytokine secreted by $CD4^+$ cells. The finding that IFN-γ mRNA expression levels increase 4 h after a PDT session is consistent with our immunohisto[illegible] ical results showing that the $CD4^+$ cell [illegible] increases 4 h after this treatment. We can s[illegible]st that PDT may activate or augment local cell[illegible] diated immunity, and increased IFN-γ levels m[illegible] promote antiviral immunity. O[illegible]ther hand, our findings also show that IF[illegible] expression levels returned to pre[illegible]tment [illegible]els 24 h after a PDT session, suggesting that the upregulation of IFN-γ induced by PDT is not sustained but transient. $CD8^+$ cells did not change significantly in number, distribution, or mor[illegible] 4 or 24 h after a PDT session, sugge[illegible] that t[illegible] immunosuppressive effect of CD[illegible]ells is not weakened within 24 h.

In this study, we used immu[illegible]chemistry to evaluate and co[illegible]e CD1[illegible]DCs in healthy foreskin tiss[illegible]nd in condyl[illegible] acuminata tissue. Th[illegible]sults [illegible]aled a [illegible] individual $CD123^+$ p[illegible] in th[illegible]perfici[illegible] dermis of lesioned t[illegible]ue, [illegible]e was [illegible]rved in healthy fore[illegible] tissue[illegible]tati[illegible]ly significant dif[illegible]ce was fo[illegible]ween the two. Imm[illegible]hemistry w[illegible]mployed to evaluate [illegible]nges [illegible] distribution of $CD123^+$ pDCs in condyloma[illegible]minata tissue before a P[illegible]ion and 4 a[illegible]h after treatment. T[illegible] results showed an increase in $CD123^+$ [illegible] numbers a[illegible]r treatment, suggesting that [illegible]23+ pDCs [illegible] a role in the immune re[illegible]a[illegible] activat[illegible]y PDT. Subsequently, qRT-PC[illegible] to the analysis of changes in expr[illegible]of IFN-α, IFN-β, ISG-15, Mx-2, TLR9, and IRF7. The results showed that in [illegible]atients, IFN-α, IFN-β, ISG-15, Mx-2, [illegible]and IRF7 expression levels gradually increased after a PDT session, suggesting that pDCs, IFN, and the MyD88 pathway may participate in this process. Their functioning may be due to massive necrosis and apoptosis of

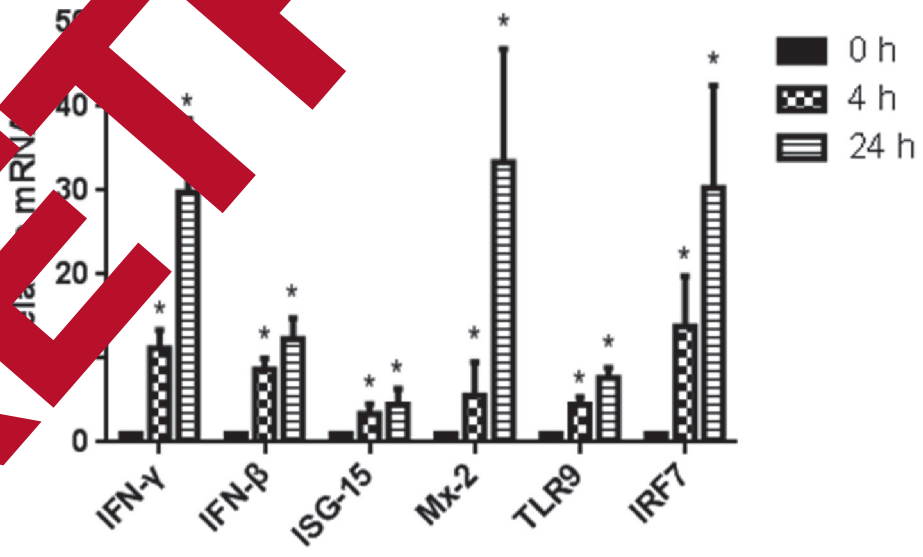


Figure 7. Relative expression levels of IFN-α, IFN-β, ISG-15, Mx-2, TLR9, and IRF7. $^{*}p < 0.05$ compared with untreated lesion tissue.

Table III. Correlation between PDT clinical efficacy and the increased levels of IFN-α and IFN-β.

Group	Number of patients	Average cure times	*p*
IFN-α, IFN-β significantly increased	5	4 ± 0.71	
IFN-α, IFN-β not significantly increased	3	6 ± 1.00	

HPV-infected keratinocytes after PDT releases large amounts of HPV viral dsDNA, which activates pDCs and is recognized and bound by TLR9 in pDCs, activating the MyD88 pathway and resulting in the production of large quantities of IFN. The latter exerts antiviral and immunostimulatory actions.

In summary, after a PDT session, the number of $CD123^+$ pDCs gradually increases and the expression of IFN-α, IFN-γ, ISG-15, Mx-2, TLR9, and IRF7 also gradually increases. These two similar trends are suggestive of a correlation between the two phenomena.

The comparison of $CD1a^+$ immunohistochemical results in condylomata acuminata tissues before and after PDT treatment reveals that $CD1a^+$ DCs are primarily distributed among epidermal prickle cells or basal cells before PDT, with few cells in the dermis. In contrast, at 4 h after a PDT session, the number of these cells in the epidermis decreases without a significant change in the number or distribution in the dermis. At 24 h after a PDT session, the number of cells further decreases across the epidermal layer with an increase in the dermis, and the dendritic protrusions of the cells diminish or completely disappear, resulting in a spindle-like morphology. These results are consistent with those reported abroad. The changes in DCs after PDT may be due to the strengthening and improvement of local specific immune responses by PDT, wherein the previously impaired immune function and the inhibited DCs may be activated. We can consider that after PDT sessions, massive necrosis and apoptosis of HPV-infected keratinocytes release large amounts of viral particles and HPV dsDNA, which activate the inhibited and impaired DCs in the epidermis and restore their ability to recognize, take up, and present antigens. Subsequently, LCs gradually migrate and accumulate in the dermis and then continuously migrate into draining lymph nodes to perform their immune functions.

Conclusions

The above results confirm that PDT for condylomata acuminata has local immunostimulation, which is represented by gradually increased levels of IFN-α and IFN-β 4 to 24 h after treatment. Our results reveal that the increased IFN-α and IFN-β levels are associated with the efficacy of PDT, such that patients with increased IFN-α and IFN-β have better clinical outcomes. To some extent, this study confirms known immunological effects of PDT. Nevertheless, the number of patients in this study is relatively small, and a bigger sample size should draw more reliable and accurate conclusions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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