Journal of Dermatological Science 54 (2009) 121–139

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.intl.elsevierhealth.com/journals/jods

Letter to the Editor

Local hyperthermia could induce migrational maturation of Langerhans cells in condyloma acuminatum

ARTICLE INFO

Keywords:
Condyloma acuminatum, Langerhans cells;
Hyperthermia

Local hyperthermia at the fever range was able to promote the migration and activation of Langerhans cells (LCs) in mice [1,2]. Local hyperthermia was shown to be clinically effective in the treatment of human papillomavirus (HPV) infected skin [3]. Cell-mediated immunity was responsible for the eradication of HPV infected keratinocytes and regression of the warty lesions [4]. Here, we modified a skin organ culture protocol [2] on condyloma acuminatum (CA), and investigated the effects of above fever range local hyperthermia on the migration and maturation of epidermal LCs, exploring the possible involvement of LCs in immune responses against HPV infection.

Biopsy materials were obtained from CA patients in the pudendum. Skin specimens from patients undergoing genital plastic surgery were obtained as normal controls upon informed consent. We designed and manufactured a local hyperthermia generator with a far-infrared emitting source and an adjustable output surface area. The heat generated by the device acted on the skin surface without direct contact. The surface temperature of skin could be controlled and stabilized at the desired degrees with an accuracy of ±0.1 °C. Approximately 1 cm × 1 cm of sterile CA and normal skin specimens were cut into four equal portions. One piece was snap frozen and stored and the remaining three pieces were separately placed in culture dishes, with dermal side down in the media (RPMI 1640), then were exposed to local heating at surface temperatures of 37, 42 and 45 °C, respectively, for 30 min. At the end of hyperthermia, the specimens were processed for immunohistochemical staining. In another set of experiment, after local hyperthermia at different temperatures, specimens were fully submerged in culture media and incubated at 37 °C for 12 h with 5% CO_2, then were taken and processed for immunohistochemical staining. In addition, the *émigrés* in culture media were collected for further study.

After local heating at 37 °C, the numbers ($\bar{x} \pm \boldsymbol{s}$) of $CD1a^+$ positive LCs in the epidermis of normal skin and CA were $782.4 \pm 114.9\ mm^{-2}$ ($n = 10$) and $692.3 \pm 141.4\ mm^{-2}$ ($n = 10$), similar to the untreated fresh specimens (data not shown). Immediately after local heating at 42 and 45 °C, the numbers of LCs in normal skin were 649.4 ± 119.4 and $510.9 \pm 118.6\ mm^{-2}$, respectively and those in CA were 535.7 ± 161.5 and $438.4 \pm 194.3\ mm^{-2}$, respectively. Either in normal skin or CA, local hyperthermia at 45 °C was significantly more effective in reducing the numbers of LCs than that of 42 °C ($p < 0.01$). At each given temperature, the numbers of LCs which remained in CA were less than those in normal skin ($p < 0.01$). As shown in Fig. 1, the numbers of $CD1a^+$ LCs were further decreased in the epidermis of both normal skin and CA, after local hyperthermia plus subsequent incubation at 37 °C for 12 h. At each given temperature, the numbers of LCs which remained in CA were less than those in normal skin ($p < 0.01$).

Cells that migrated into the culture media were collected and double stained with mouse anti-human FITC-labeled CD1a and PE-labeled CD83 mAbs, a molecule that stably expressed on activated DCs [5] (all mAbs were from BD PharMingen, USA), then were analyzed with flow cytometry. As shown in Table 1, the proportions of $CD1a^+/CD83^+$ LCs were significantly increased in *émigrés* from both normal skin and CA at higher temperatures. At each given temperature, there was more $CD1a^+/CD83^+$ LCs in *émigrés* from CA than normal skin.

Increased expression of CCR7 and decreased expression of CCR6 on LCs are required for their functional migration to regional lymph nodes [6,7]. To explore the influence of hyperthermia on CCR6 and CCR7 expression, we extracted total RNA from MACS® (Miltenyi Biotec) purified $CD1a^+$ LCs by using an RNA extraction kit (QIAGEN). The real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed using a Rotor Gene3000 Detector System (Corbett Research, Australia). In $CD1a^+$ LCs from *émigrés* of normal skin ($n = 5$), the expressions of CCR6 mRNA were decreased by 30% and 60% after being exposed to 42 and 45 °C, respectively, as compared with that of 37 °C ($p < 0.01$). While in *émigrés* from CA ($n = 5$), the expressions of CCR6 mRNA were reduced by 50% (42 °C) and 90% (45 °C), respectively ($p < 0.01$). In contrast, in $CD1a^+$ LCs from *émigrés* of normal skin ($n = 5$), the expressions of CCR7 mRNA were increased by 2- and 5-fold after being exposed to 42 and 45 °C, respectively, as compared with that of 37 °C ($p < 0.05$). While in *émigrés* from CA ($n = 5$), the expressions of CCR7 mRNA were increased by 3-fold (42 °C) and 19-fold (45 °C), respectively ($p < 0.01$).

Due to the limited numbers of $CD1a^+$ LCs in the *émigrés*, we were unable to quantify the expression of CCR6 and CCR7 proteins on $CD1a^+$ LCs.

Hyperthermia temperature at 43 °C is regarded as a breakpoint, beyond which more severe cell apoptosis occurs [8]. Our previous clinical trial showed that local hyperthermia temperatures at 42 and 45 °C were effective in treating the warts, the effective rates tended to be better with the latter (Gao XH, unpublished observation).

We observed that local hyperthermia at 42 and 45 °C, especially the latter, promoted the migration and maturation of LCs in normal human skin. However, these effects were more pronounced in CA, as manifested by higher proportions of $CD1a^+/CD83^+$ LCs in the *émigrés*.

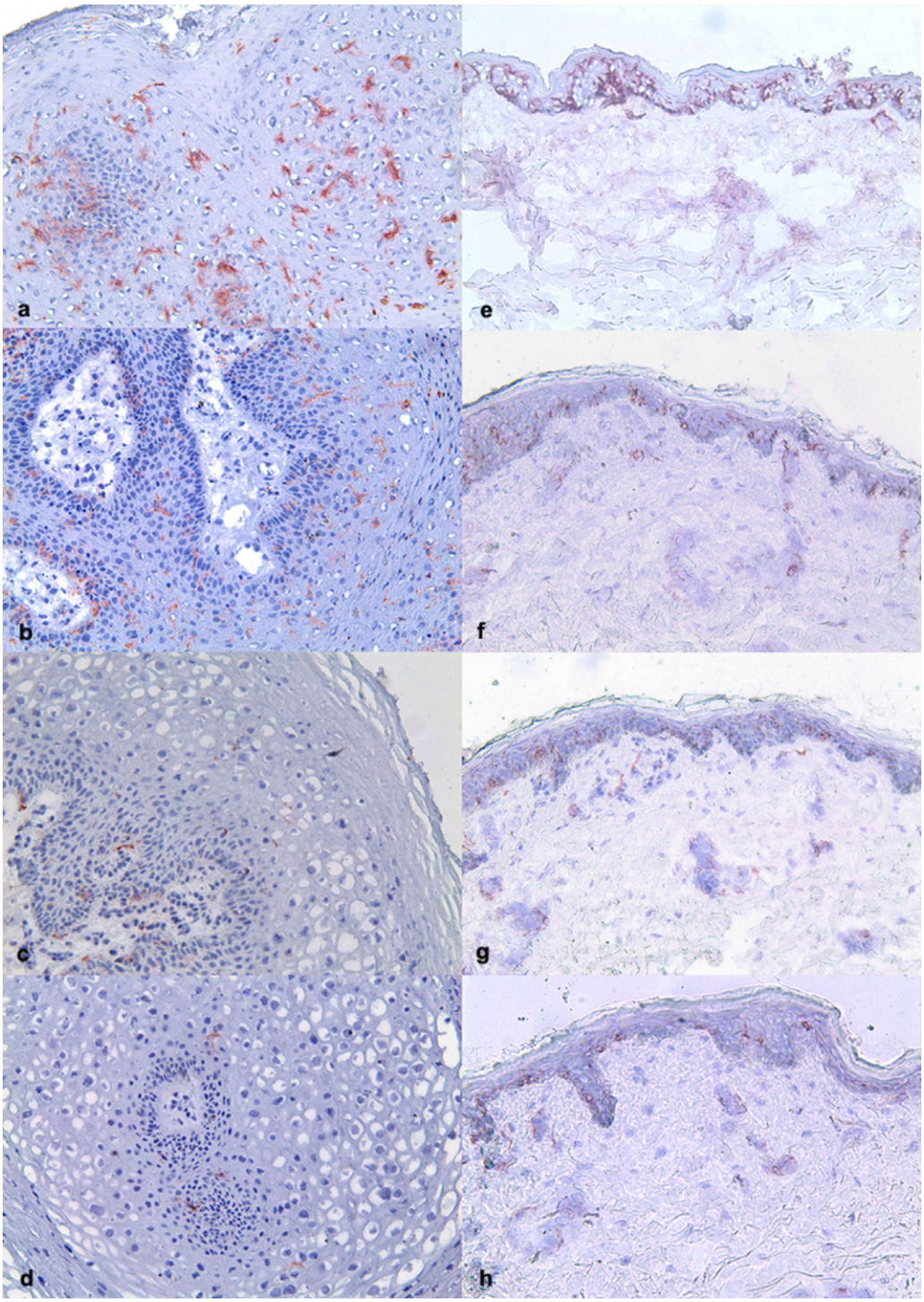

Fig. 1. CD1a$^+$ LC in CA (a) and normal skin (e). Representative figures of remaining CD1a$^+$ LCs in CA (left column, $n = 10$) and normal skin (right column, $n = 10$) after local hyperthermia with different temperatures plus incubation for 12 h; (b–d) local hyperthermia at 37 °C (692.3 ± 141.4 mm^{-2}), 42 °C (535.7 ± 161.5 mm^{-2}), and 45 °C (438.4 ± 194.3 mm^{-2}), respectively; (f–h) local hyperthermia at 37 °C (782.4 ± 114.9 mm^{-2}), 42 °C (649.4 ± 119.4 mm^{-2}), and 45 °C (510.9 ± 118.6 mm^{-2}), respectively. Numbers in parentheses were represented as $\bar{x} \pm \boldsymbol{s}$. The differences between numbers of the epidermal CD1a$^+$ LCs in normal skin and CA at the same temperature were analyzed by independent t-test (all $p < 0.01$). Magnification: 200×.

Ostberg illustrated that fever range temperatures could increase the numbers of DCs in the migrated population, and enhance the migration-associated functional maturation of these DCs in mice [2]. We found that above fever range hyperthermia had similar effects. Further, local hyperthermia at 42 and 45 °C could concomitantly increase the expression of CCR7 and decrease the expression of CCR6, which are prerequisite for LCs to reach regional lymph node [9]. This scenario implied that there might be an increased influx of LCs to draining lymph nodes upon local hyperthermia. Danno and Sugie observed that near-IR could reduce the number of LCs in the epidermis of the mice and, subsequently ameliorated the intensity of allergic contact dermatitis [10]. There is a need to further test the role of local hyperthermia on specific immune response against HPV-specific antigens.

Table 1
LCs in *émigrés* from normal skin and CA ($\bar{x} \pm s$).

Surface markers of LCs	Temperature	Normal skin ($n = 5$)	CA ($n = 5$)	p
$CD1a^+$ (%)	37 °C	0.67 ± 0.26	1.22 ± 0.28	<0.05
	42 °C	1.17 ± 0.20	3.14 ± 0.16	<0.01
	45 °C	2.25 ± 0.36	4.71 ± 1.11	<0.01
p		<0.01	<0.01	
$CD1a^+/CD83^+$(%)	37 °C	0.59 ± 0.08	0.68 ± 0.25	0.471
	42 °C	1.14 ± 0.17	2.37 ± 1.17	<0.05
	45 °C	1.67 ± 0.54	4.44 ± 0.48	<0.01
p		<0.01	<0.01	

The significances of differences between the numbers of $CD1a^+$ and $CD1a^+/CD83^+$ LCs in *émigrés* subjected to different temperatures were analyzed by repeated measures analysis of variance (ANOVA) and, the significances of differences between the numbers of $CD1a^+$ and $CD1a^+/CD83^+$ LCs in *émigrés* from normal skin and CA at the same temperatures were analyzed by independent *t*-test.

Acknowledgments

This work was supported by grants from Program for Changjiang Scholars and Innovative Research Team in University (IRT0760), National Natural Science Foundation of China (No. 30740082), and Program for New Century Excellent Talents in University (NCEP-04-028).

References

[1] Ghaznawie M, Papadimitriou JM, Heenan PJ. The repopulation of murine Langerhans cells after depletion by mild heat injury. Br J Dermatol 1999; 141:206–10.
[2] Ostberg JR, Kabingu E, Repasky EA. Thermal regulation of dendritic cell activation and migration from skin explants. Int J Hyperthermia 2003;19: 520–33.
[3] El-Tonsy MH, Anbar TE, El-Domyati M, Barakat M. Density of viral particles in pre and post Nd: YAG laser hyperthermia therapy and cryotherapy in plantar warts. Int J Dermatol 1999;38:393–8.
[4] Hong K, Greer CE, Ketter N, Van Nest G, Paliard X. Isolation and characterization of human papillomavirus type 6-specific T cells infiltrating genital warts. J Virol 1997;71:6427–32.
[5] Prechtel AT, Steinkasserer A. CD83: an update on functions and prospects of the maturation marker of dendritic cells. Arch Dermatol Res 2007;299: 59–69.
[6] Saeki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 1999;162:2472–5.
[7] Annels NE, Da Costa CE, Prins FA, Willemze A, Hogendoorn PC, Egeler RM. Aberrant chemokine receptor expression and chemokine production by Langerhans cells underlies the pathogenesis of Langerhans cell histiocytosis. J Exp Med 2003;197:1385–90.
[8] Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, et al. The cellular and molecular basis of hyperthermia. Crit Rev Oncol Hematol 2002;43:33–56.
[9] Fleming MD, Pinkus JL, Fournier MV, Alexander SW, Tam C, Loda M, et al. Coincident expression of the chemokine receptors CCR6 and CCR7 by pathologic Langerhans cells in Langerhans cell histiocytosis. Blood 2003;101: 2473–5.
[10] Danno K, Sugie N. Effects of near-infrared radiation on the epidermal proliferation and cutaneous immune function in mice. Photodermatol Photoimmunol Photomed 1996;12:233–6.

Xiaodong Li[a,b], Xing-Hua Gao[a,*], Li Jin[c], Yakun Wang[a], Yuxiao Hong[a], Uwesu Omari Mchepange[a], Xiaoqin Wang[a], Yi Jiang[a], Huachen Wei[ad], Hong-Duo Chen[a]

[a]*Department of Dermatology, No. 1 Hospital of China Medical University, 155N. Nanjing Street, Shenyang 110001, China*
[b]*Department of Dermatology, Fengtian Hospital Affiliated to Shenyang Medical College, China*
[c]*Department of Gynecology, No. 1 Hospital of China Medical University, Shenyang 110024, China*
[d]*Department of Dermatology, Dermatology Research Laboratories, Mount Sinai Medical Center, New York, NY 10029, United States*

*Corresponding author. Tel.: +86 24 8328 2326; fax: +86 24 8328 2633
E-mail address: gaobarry@hotmail.com (X-H. Gao)

8 August 2008

doi:10.1016/j.jdermsci.2008.12.004

Letter to the Editor

Silencing the androgen receptor: New skills for antiandrogen oligonucleotide skin and hair therapy

ARTICLE INFO

Keywords:
Antiandrogen oligonucleotides; Skin and hair; Cutaneous therapy

Hair growth and follicular cycle are regulated predominantly through androgens under complex genetic and hormonal control. Human hair growth occurs in cycles of three phases, anagen (continuous growth), catagen (cessation of growth) and telogen (resting phase). In genetically susceptible subjects, hair follicles in vertex and frontal regions of the scalp respond to androgens by reducing length of the anagen phase and regression of the follicles producing weaker and thinner hairs. More than 50% of men by the age of 50 years and women over 60 years suffer from androgenetic alopecia. The mesenchymal-derived dermal papilla cells (DPC) exert a control on the hair growth cycle, express androgen receptor, are responsive to androgen hormones and are implicated in triggering baldness in humans. Today, androgens are considered inhibiting hair follicle activity by early inducing catagen. Although the pathogenic mechanisms underlying androgenetic alopecia are not fully understood it is now accepted that androgens (mainly testosterone and dihydrotestosterone) inhibit hair follicle activity, probably by triggering the expression or repression of some genes (not yet identified) involving modification of DPC and epithelial cells relationship, thereby leading to the induction of programmed cell death. Consequently, the anagen length period shortens, favoring catagen and finally miniaturizing the follicle and thinning the hair [1,2]. Moreover, other skin disorders such as acne vulgaris, hirsutism and seborrhea are dependent or sensitive to the androgens action [3,4]. With this in view, the developing of new topical therapeutics