Preliminary Report

Co-Stimulatory Molecules on Peripheral Blood Mononuclear Cells and Tissue Infiltrating Cells of Skin Wart and In Vitro Poke Weed Mitogen Stimulation

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Background: *Skin wart is a lesion caused by human papilloma viruses (HPVs) that can infect both male and female.*
Objective: *Quantify the number of $CD28^+$, $CD86^+$, $CD152^+$ and $\gamma\delta^+$ in peripheral blood mononuclear cells (PBMCs) of subjects with skin wart. Identify $CD86^+$ and $\gamma\delta^+$ cells in skin wart cryosections.*
Material and Method: *Sixteen subjects with skin warts on face, hand, finger, knee, foot or plantar, both male and female, aged between 19-59 years-old, were recruited from Ramathibodi Hospital, Mahidol University, Bangkok*
Results: *CD86 and CD152, on peripheral blood mononuclear cells (PBMCs) of subjects with skin wart are significantly lower compared to controls. Tissue cryosection staining for $CD86^+$ and $\gamma\delta^+$ cells showed no difference among subjects with skin wart and control. Proliferative response to poke weed mitogen of subjects with skin wart is significantly lower than control subjects.*
Conclusion: *There was no difference in the number of subjects positive for CD28 and $CD86^+$ cell between normal and skin wart subject, but an increase in skin wart subjects with $\gamma\delta^+$ cells.*

Keywords: *Skin wart, Co-stimulatory molecule, Immune cell competency*

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Skin wart is a lesion caused by human papilloma viruses (HPVs) that can infect both male and female. The HPVs infect mucosal and keratinized epithelium with each type of HPV selectively infecting different anatomical sites[1,2]. Warts appear to occur more frequently in immunosuppressed patients, especially in cell-mediated immunity such as in leukemia, lymphoma and renal transplantation patients[3,4]. In addition, the spontaneous regression and/or persistence of warts are related to the immunologic responsiveness of the individual[5].

Engagement of T-cell receptor (TCR) with peptide-MHC alone is insufficient to activate specific mature na□ve T cell[6]. The most critical co-stimulatory molecule on T cell is CD28, which binds to either B7-1 (CD80) or B7-2 (CD86) expressed on antigen presenting cells (APCs)[7]. Surface CTLA-4 (CD152) expressed on T cells is known to terminate T cells responses by activation of inhibitory signal [8-9], while CD28 delivers signals that enhance T cell response[10,11]. However, the affinity with which CD152 binds with co-stimulatory molecules (CD80 and CD86) is higher than that of CD28[12,13]. In cutaneous immune response, $\gamma\delta^+$ immune cells function by releasing cytokine, demonstrating immunoregulatory activity and direct cytolysis[14,15].

The aim of the present study was to quantify the number of $CD28^+$, $CD86^+$, $CD152^+$ and $\gamma\delta^+$ in peripheral blood mononuclear cells (PBMCs) of subjects with skin wart and to identify $CD86^+$ and $\gamma\delta^+$ cells in skin wart cryosections by immunocytochemistry staining technique. The proliferative response of PBMCs upon poke weed mitogen stimulation was also investigated.

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Material and Method

Subjects

Sixteen subjects with skin warts on face, hand, finger, knee, foot or plantar, both male and female, aged between 19-59 years-old, were recruited from Ramathibodi Hospital, Mahidol University, Bangkok. All subjects, including controls signed the consent form approved by the ethic clearance committee of Ramathibodi Hospital.

Preparation of peripheral blood mononuclear cells

PBMCs from skin wart and control subjects were separated from heparinized blood by density gradient centrifugation using the modified method of Boyum[16]. PBMCs were adjusted to the desired concentration (2.5 x 10^6 cells/ml) with 10% FBS RPMI 1640 medium (Sigma Chemical Co. St. Louis, MO, USA).

Tissue biopsies

Skin wart and normal skin biopsies were immediately embedded in tissue embedding medium (Jung, Germany) and stored at -70°C. The frozen tissues were sliced into 6 μm sections in a microtome (Tissue TEK II, Mile, USA) and placed on silane-coated slides.

Immunocytochemical staining

Approximately 1 x 10^5 PBMC in 200 μl of phosphate-buffered saline were sedimented onto a clean glass slide using cytobucket trunnion carrier (Jouan, France) and stained with monoclonal antibodies against CD28, CD86, CD152 and γδ chains (Immunotech, France) by using the streptavidin-biotin (Immunotech, France) detection technique. Endogenous peroxidase activity and non-specific binding were blocked according to the manufacture's instruction. Diaminobenzidine (DAB) was used as the chromogen and the cells were counterstained with hematoxylin. Quantization of positive stained cells was performed by counting at least 200 mononuclear cells under light microscope (x100 objective).

Frozen tissue slides were allowed to reach room temperature and then endogenous peroxidase and protein blocking solution was applied before staining with monoclonal antibodies against CD86 and γδ as described above.

Proliferation assay

PBMCs (2.5 x 10^5 cells/well) were treated (in triplicate wells) with 10 μg/ml poke weed mitogen (PWM) (Sigma USA) in 96-well flat bottom tissue culture plate, at 37°C, in 5% CO_2 incubator for 72 hr. Then 0.5 μCi of ^3H thymidine (specific activity 74.0 GBq/mmol, 2.0 Ci/mmol, Amersham Pharmacia Biotech, UK) were added per well and the sample incubated for 18 hr. Cells were harvested onto glass fiber filter using cell harvester (PHD Cell Harvester, USA) and ^3H thymidine incorporation was measured in a β-counter (Packard Bioscience, USA).

The stimulation index (SI) was calculated as follows:

$$SI = \frac{\text{mean count per minute following PWM stimulation}}{\text{mean count per minute without PWM stimulation}}$$

SI value of two or more was considered as being positive proliferation.

Statistical analysis

Data were analyzed by Mann-Whitney U-test; p-values less than 0.05 are considered significant.

Results

Quantitation of $CD28^+$, $CD86^+$, $CD152^+$ and $\gamma\delta^+$ cells in peripheral blood mononuclear cells

Percents of $CD86^+$ and $CD152^+$ cells from patients with skin wart are significantly lower than that of control subjects in Table 1. However, there are no differences of $CD28^+$ and $\gamma\delta^+$ PBMCs between the two groups of subjects. Staining of $CD86^+$ cells in PBMCs is shown in Fig. 1.

Detection of $CD86^+$ and $\gamma\delta^+$ cells in skin wart cryosection

$CD86^+$ cells could be detected in three samples from sixteen skin biopsies (19%) while in normal skin one of six samples (17%) was detected. On the other hand, $\gamma\delta^+$ cells could be detected in two of six normal skin biopsies (33%) and in one of sixteen warts (6%).

Table 1. Percent $CD28^+$, $CD86^+$, $CD152^+$ and gd^+ T cells from subjects with skin wart and controls

Phenotype	Percent ± SD	
	Control (n = 15)	Skin wart (n = 14)
CD28	19.2 ± 2.3	17.9 ± 3.4
CD86	19.1 ± 2.0	13.0 ± 3.0*
CD152	10.1 ± 1.8	8.0 ± 1.7*
$\gamma\delta^+$	9.3 ± 2.0	8.0 ± 3.0

* $p < 0.05$

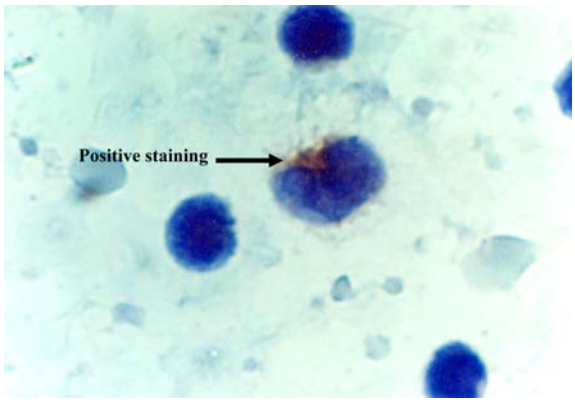

Fig. 1 Staining of $CD86^+$ cells (arrow) in peripheral blood mononuclear cells (x100 magnification)

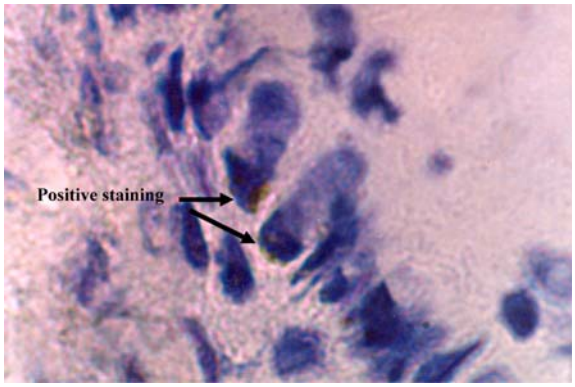

Fig. 2 Staining (arrow) of $\gamma\delta^+$ cells in cryosection (x100 magnification)

Staining of skin cryo-section for $\gamma\delta^+$ cell was shown in Fig. 2.

Proliferative response to poke weed mitogen stimulation

The stimulation index (SI) of proliferative response to poke weed mitogen stimulation for subjects with skin wart (22 ± 4, n = 14) is significantly lower than that for control (33 ± 3, n = 15) ($p < 0.05$). SI ratio of common antigen to tetanus toxoid was over 20 in both groups.

Discussion

The present study showed a reduction in co-stimulatory ($CD86^+$) and counter molecules ($CD152^+$) expressions on PBMCs from subjects with skin wart compared with control subjects, suggesting a decrease of immune cell activity. This was supported by the decrease of proliferative response to PWM mitogen stimulation in skin wart subjects. Percent $CD152^+$ cell of the control group in the present study was similar to that reported by others[17]. Thus, the reduction in $CD152^+$ cells from skin wart subjects may indicate a decrease in the number of activated T cells as this marker is expressed on such activated cells[18].

The local skin immune status was studied by monitoring the presence of $CD86^+$ cells and $\gamma\delta^+$ cells in cryosections. There was no difference in the number of subjects positive for $CD86^+$ cell between normal and skin wart subject in this study, but the increase in skin wart subjects with $\gamma\delta^+$ cells is consistent with the demonstration of regulatory role of $\gamma\delta^+$ cells in cutaneous immune response[19-21].

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Co-stimulatory molecules บนผิวเม็ดเลือดขาวชนิดนิวเคลียสเดี่ยวและเซลล์ที่อยู่บริเวณชิ้นเนื้อในผู้ป่วยหูด และบทบาทของเซลล์เม็ดเลือดขาวชนิดนิวเคลียสเดี่ยวเมื่อถูกกระตุ้นด้วยสารโพควีดไมโตเจน

พจมาน ผู้มีสัตย์, สมยศ จารุวิจิตรรัตนา, มลวิภา วงษ์สกุล

เปอร์เซ็นต์ของ Co-stimulatory molecule; CD86 และ CD152 บนเซลล์เม็ดเลือดขาวชนิดนิวเคลียสเดี่ยวของผู้ป่วยหูดลดลงอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุม แต่ไม่พบความแตกต่างของ $CD28^+$ และ $\gamma\delta^+$ เซลล์ระหว่างผู้ป่วยหูดและกลุ่มควบคุม ชิ้นเนื้อที่ย้อม $CD86^+$ และ $\gamma\delta^+$ เซลล์มีเปอร์เซ็นต์ไม่แตกต่างกันระหว่างผู้ป่วยหูดและกลุ่มควบคุม การเพิ่มจำนวนเซลล์เมื่อถูกกระตุ้นด้วยสารโพควีดไมโตเจน พบว่าในผู้ป่วยหูดมีการลดลงอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุม