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Iterleukin-4 and interferon-γ in allergic contact dermatitis with atopic background in leather tannery factory worker

S Widhiati[1], AS Siswati[1*], H Kariosentono[2]
[1]Department of Dermatovenerology, Faculty of Medicine Gadjah Mada University, Yogyakarta,
[2]Department of Dermatology, Faculty of Medicine Sebelas Maret University, Surakarta, Indonesia

ABSTRACT

Immune response in allergic contact dermatitis (ACD) patient is dominated by T helper-1 (Th-1) response characterized with increase in interferon gamma (IFN-γ). However, in atopic individual, the immune response is dominated by T helper-2 (Th-2) response which characterized with the presence of interleukin-4 (IL-4). Based on that condition, it is hypothesized that atopic individual was hardly to develope ACD. In leather factory, many workers are prone to develope ACD. The aim of this study is to differentiate the cytokine profiles of IL-4 and IFN-γ of ACD patients with or without atopic background. Using a cross-sectional design, this study involved 30 subject assigned into two groups, one group consisted of 15 subjects with ACD who had atopic background (ACD atopic), the other group consisted of 15 subjects with ACD who had no atopic background (ACD non atopic). Both groups were examined by patch test and confirmed to have ACD when the result was minimally + 1 in 48 and/or 96 hours examination. Atopic skin diathesis score ≥8 was used to determine the possibility of having atopic background. Serum IL-4 and IFN-γ concentration were determined using ELISA. Data were analyzed using SPSS with *Mann-Whitney* non- parametric test. The results showed that the mean value of IL-4 in both groups were 0.18 ± 0.14 pg/mL and 0.25 ± 0.29 pg/mL (p = 0.917) whereas the mean value of IFN- γ in both groups were 13.03 ± 23.90 pg/mL and 2.76 ± 5.67 pg/mL, (p = 0.096). In conclusion, the cytokine profiles of IL-4 and IFN-γ were not significantly different between ACD atopic and ACD non atopic individuals. This finding suggested that atopic and non-atopic individuals had a similar immunologic response during development of ACD.

Key words: immunologic response-cytokine-occupational contact dematitis-T helper-patch testing

INTRODUCTION

Occupational contact dermatitis is one of the main problems in occupational dermatoses. It occurs in 90-95% of occupational dermatoses.[1] In leather and tannery industry, workers are frequently exposed to sensitizing chemical during their work in pre-tanning, tanning and dyeing, fat liquoring and finishing.[2] Potassium dichromate is the most frequent allergen found in leather worker in Buenos Aires.[3] Hence, in such condition many workers suffer from allergic contact dermatitis (ACD).

The prevalence of ACD in atopic patients is still unknown. Several studies suggested that ACD is less frequent in atopic patients compared to non atopic patients.[4,5] However others argued that atopic patients are more prone to have ACD compared to non atopic patients.[6]

Allergic contact dermatitis is a delayed type hypersensitivity reaction. The T helper-1 (Th-1) is important in sensitization and elicitation reaction which mainly express interferon gamma (IFN-γ) and interleukin-2 (IL-2). Atopic individuals have Th-2 reaction shortly after birth. Since cytokines produced by Th-2 can suppress the differentiation of CD4 T cell to Th-1, it was suspected that the ability of atopic dermatitis (AD) patients to develope contact hypersensitivity reaction would be diminished. This hypothesis was proved by Rysedt[4] who showed that

* corresponding author: *asrisiswati@yahoo.com*

patients who had atopic background had lower probability than patients who had no atopic background to develop allergic dermatitis.

Interferon-gamma expression correlates with severity of ACD, because of it's function as pro inflammatory cytokine.[7] However, Zedan *et al.*[8] did not find the correlation between increment of serum IFN-γ level with severity of ACD. In AD, the serum IFN-γ level also not correlate with severity of disease.

In vitro studies has proven the role of cytokine in ACD. However, *in vivo* study in human disease showed that the role of cytokine in ACD remains unclear. Therefore, it is important to evaluate the different level of Th-1 and Th-2 cytokines in ACD patient in order to know the role of cytokine *in vivo*. This study was conducted to investigate serum level of Th-1 cytokine (IFN-γ) and Th-2 cytokine (IL-4) in ACD patients who were atopic and non atopic in order to asses the different immune response mechanism of both groups.

MATERIALS AND METHODS

Subjects

This study included all leather and tannery worker with occupational ACD (n=15) that were diagnosed according to Mathias criteria.[9] Atopic skin diathesis (ASD) were recorded based on Diepgen and Coenraads 2000.[10] Atopic skin diathes was established when the score was $\geq$ 8. All subject did not take antihistamine or corticosteroid oral 2 weeks prior study, did not have influenza or granulomatous skin diseases, diabetes mellitus, and pregnancy. In addition, 15 ACD workers without history of ASD were included as a control group. The study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University, Yogyakarta.

Patch testing

Patch test was performed in all subjects with 22 European Standard Series, as well as with standard shoes series and suspected personal items. The substances were applied to the back for 2 days with Finn Chamber (Epitest Ltd. Helsinki Finland), and readings were performed at 2, 4, and 7 days after application using recommendations of International Contact Dermatitis Research Group (ICDRG). Testing was not performed if dermatitis was present on the back, or if severe dermatitis existed elsewhere. Diagnosed of ACD was established if subject had positive patch test result with suspected substances from the work environment.

Measurement of serum cytokine level

Two mL of venous blood was collected, and allowed to clot in room temperature for 30 minutes, then centrifuged for 10 minutes in 1000 x g. Sera were stored in -80°C, and thawed immediately prior to analysis. Quantification of IL-4 and IFN-γ (Biolegend-Max®-San Diego,US) was performed using Enzyme-linked immunosorbent assay (ELISA). All determination were performed in duplicate. Minimal detection assay was 0.0 pg/mL, bellow detection limit was signed to zero.

Statistical analysis

Data were analyzed using Mann-Whitney U-test. Results were considered significant if $p<0.05$.

RESULTS

Thirty ACD subjects (20 men and 10 women) were divided into 2 groups, one group consisted of 15 subjects (11 men and 4 women) with ACD who had atopic background (ACD-atopic), the other group consisted of 15 subjects (9 men and 6 women) with ACD who had no atopic background (ACD-non atopic). The subjects mean age was 39 years old (range from 22 to 65 years olds). All subjects worked for 48 hours/week, and 6 work days. They had been working in the factory averagely for 81 months for the ACD–atopic subjects and 134 months for the ACD-non atopic subjects.

Seventeen subjects worked in wet area (pre-tanning and tanning), 13 subjects worked in dry area (finishing). To minimize bias from endogen and exogen confounding factor, homogen test was conducted and the results showed that there was no signicant difference of subjects characteristics between subjects with ACD-atopic and ACD-non atopic ($p>0.05$) as shown in TABLE 1.

TABLE 1. Subject characteristic based on homogenity test of age, gender, duration of work, and type of work

Characteristic	Allergic contact dermatitis		Total	p
	ACD-atopic	ACD-non atopic		
Age (years)				
• mean	38,73	41,0		0,18
• SD	13,63	8,98		
Gender				
• Men	11(36,7%)	9(30%)	20 (66,7%)	0,44
• Women	4(13,3%)	6 (20%)	10 (33,3%)	
Work Duration (months)				
• mean	81,67	134		0,56
• SD	21,48	24,13		
Type of work				
• wet	9 (30%)	8 (26,6%)	17 (56,6%)	0,52
• dry	6 (20%)	7 (23,3%)	13 (43,3%)	

All subjects had at least one positive patch test result, and had history contact with suspected substances from environment. The substances were diphenil tiourea, 4-aminobenzene, n,n difenilguanidine, potassium dikromat, 4-penilendiamine, tiuram mix, 2-n-octyl-4-isothiazolin-3-one, 4-tert-butylphenol fromaldehide resin, and primin.

Serum IL-4 level in both groups was not significantly different (0.18±0.14 pg/mL for ACD atopic compared to 0.25±0.29 pg/mL for ACD non atopic, p>0.05). So was serum IFN-γ level in both groups (13.03±23.90 pg/mL for ACD atopic compared to 2.76±5.67 pg/mL for ACD non atopic, p>0.05). However, mean concentration of serum IFN-γ in ACD atopic group tended to be higher than ACD non atopic group (FIGURE 1).

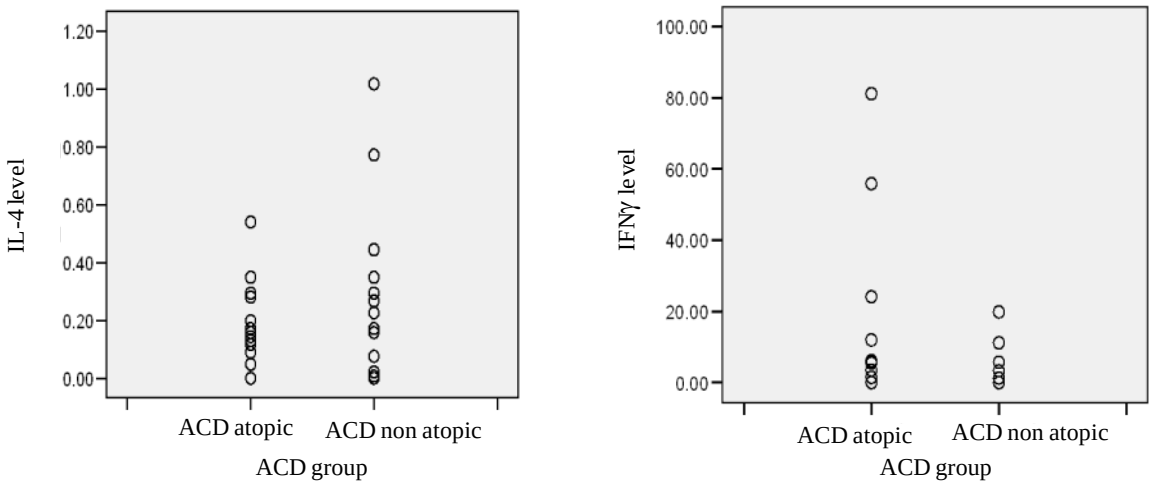

FIGURE 1. Serum IL-4 and IFN-γ level in ACD atopic group and ACD non atopic group.

DISCUSSION

The result showed that low level of IL-4 in this study was due to the absence of acute lesion (skin rash) in both groups. This finding was similar with Wittmann *et al.*[11] and Thepen *et al.*[12] studies which reported that IL-4 concentration in AD and ACD patient decreased in chronic phase. Th-2 immune response did not increase in chronic ACD, even in atopic person.

This study showed that the level of IL-4 in ACD was not different between atopic and non atopic individuals. It was thought that this result was because immune response to ACD was similar in both groups. Szeptietowski *et al.*[13] also reported the same results by inducing ACD in nickel allergic subject concomitant to AD. No significant expression of IL-5 on ACD concomitant with AD also reported by Buchvald and Lundeberg.[14] This study also did not found the role of Th-2 in ACD.

Serum IFN-γ level in ACD atopic was statistically not different with ACD-non atopic in this study. This may show that the immune response in both groups was the same. Both groups needed Th-1 induction to become ACD. Although atopic individual had a predominant Th-2 response, but when ACD occur, Th-1 response played a pivotal role.

All patients were given topical steroid at the back after patch testing was performed. Subjects also were educated to use gloves and boots when working. Rotation from wet area to dry one was also encouraged.

The limitation of this study may be due to improper time of obtaining blood samples. Blood samples were obtained 3 weeks after patch test. At this time the interleukin level already decreased and disappeared. Cytokine level in circulation was difficult to count because of low half life and transient product.[15] The reason why the author chose 3 weeks after patch test to collect blood sample was the intense exposure of occupational allergen which stimulate blood circulation to produce pro-inflammatory cytokine.

Atopic skin diathesis is one of the risk factors in occupational dermatoses.[16-18] Atopic score by Diepgen *et al.*[19] has been validated to be used in epidemiology setting. Atopic skin diathesis is a predictive factor in having AD in past, present or future. Some studies consider of that diagnosis of ASD was established if the atopic score was ≥ 10, score 8-9 was considered as a possibillity to have ASD.[16] Although the association of ASD with irritant contact dermatitis (ICD) is obvious, but the correlation of ASD with ACD is still controversial. Most studies did not find the correlation between ASD and ACD. The reason why the author used atopic score ≥ 8 to represent ASD was because in developing country prevalence of AD was lower than developed country. Based on hygiene hypothesis theory this was due to increase of sibling, lower socio-economic, and exposure with pets.[20] Subject of this study had low socio-economic status and high exposure to microbials caused by the minimal personal hygiene. Those may be the reason why ASD were not fully developed.

Some of the studies that found the role of cytokine in ACD and AD evaluated their cytokine level by using peripheral blood mononuclear cell cultured and elicited with appropriate allergen. By this technique, the confounding factors can be minimized and cytokine assay can be performed easily. Niwa *et al.*[21] also found that cytokine assay using blood samples could produce lower result compared to serum or plasma sample.

Since the sample size in this study was small, the finding of this study can not be generalized to the general population of leather and tannery factory worker. However, this study suggested thatTh-1 response in atopic and non atopic individual acted similar when developed ACD. Further investigation will be required to define the role of cytokine type-1 and type 2 in development of ACD.

CONCLUSION

This study suggested that atopic and non-atopic individuals had a similar immunologic response during development of ACD, because both groups had no difference in IL-4 and IFN-γ serum level.

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