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

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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 develop ACD. In leather factory, many workers are prone to develop 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 dermatitis-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...
hypothesis was proved by Ryseck, who showed that 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 its function as pro-inflammatory cytokine. However, Zidan et al. 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 assess 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. Atopic skin diathesis (ASD) were recorded based on Diepgen and Coenraads 2000. Atopic skin diathesis was established when the score was ≥ 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 (Bilegend-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 old). 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-nonatopic subjects.

Seventeen subjects worked in wet area (pre-tanning and tanning), 13 subjects worked in dry area (finishing). To minimize bias from endogenous confounding factor, homogen test was conducted and the results showed that there was no significant 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 homogeneity test of age, gender, duration of work, and type of work

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Allergic contact dermatitis</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACD-atopic</td>
<td>ACD-non atopic</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.73</td>
<td>41.0</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>73.63</td>
<td>8.98</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>11 (36.7%)</td>
<td>9 (30%)</td>
<td>20 (66.7%)</td>
</tr>
<tr>
<td>Women</td>
<td>4 (13.3%)</td>
<td>6 (20%)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Work Duration (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>81.67</td>
<td>82.68</td>
<td>0.56</td>
</tr>
<tr>
<td>SD</td>
<td>21.48</td>
<td>24.13</td>
<td></td>
</tr>
<tr>
<td>Type of work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wet</td>
<td>9 (30%)</td>
<td>8 (26.6%)</td>
<td>17 (56.6%)</td>
</tr>
<tr>
<td>dry</td>
<td>6 (20%)</td>
<td>7 (23.3%)</td>
<td>13 (43.3%)</td>
</tr>
</tbody>
</table>

All subjects had at least one positive patch test result, and had history contact with suspected substances from environment. The substances were diphenyl thiourea, 4-aminoazdene, n,n difeniguanidine, potassium dikromat, 4-penilendianiamine, tiuram mix, 2-n-octyl-4-isothiazolin-3-one, 4-tert-butyphenol formaldehyde 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±8.67 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.\textsuperscript{1,8} and Thepen et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{6,8} Atopic score by Diepgen et al.\textsuperscript{16} 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 possibility to have ASD.\textsuperscript{17} 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 socioeconomic, and exposure with pets.\textsuperscript{19} Subject of this study had low socioeconomic 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.\textsuperscript{20} 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 that Th-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.

ACKNOWLEDGMENT

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REFERENCES

DISCUSSION

A. TAMs in Generals

The monocyte develops from a pluripotent stem cell in the bone marrow under the influence of soluble hematopoietic growth factors and by physical interactions with stromal cells as well as extracellular matrix. The monoblast, which is the earliest cell committed to the monocyte lineage, differentiates via a promonocyte into a mature monocyte, which after a short period (<24 hours), leaves the bone marrow and enters the blood stream as a quiescent (G0/G1) cell. Such monocytes may then differentiate further into resident tissue macrophages and, depending on the local microenvironment, acquire specialized phenotypic characteristics and diverse functions.

Macrophages have a pleiotropic biological role. In the setting of tumors, TAMs have a range of functions with the capacity to affect diverse aspects of neoplastic tissues including angiogenesis and vascularisation, stroma formation and dissolution, and modulation of tumor cell growth (enhancement and inhibition). When activated, they can induce neoplastic cell death (cytotoxicity, apoptosis) and/or elicit tumor destructive reactions through alteration of the number of microvasculature.

The number of macrophages is greatly (10-65%) among different tumors studied. However, the percentage of TAMs is usually maintained at a relatively stable level for a particular tumor type during transplantation into and growth in syngeneic hosts. The infiltration of mononuclear cells is generally restricted to the stromal areas with few cells infiltrating into tumor nests and between the tumor cells themselves. This migration is mediated by chemotactic factors that induce inflammatory cells to leave the vascular compartment and egress into the surrounding areas. Some tumors, however, produce inhibitors of chemotaxis. The mechanisms modulating these processes are poorly defined.

B. TAMs and Breast Cancers

Breast cancers are known to contain a high proportion of infiltrating leukocytes, including TAMs. Tumor cells attract monocytes by producing chemotactic agents, including MCP-1, M-CSF, TGF-β and undefined mediators. Tumor-derived signals can induce both pro- and anti-tumor effector in TAMs. Depending on the signal activations and susceptibility of tumor target cells, TAM can either enhance or inhibit tumor growth. As anti-tumor effector, TAMs can mediate direct anti-tumor cytotoxicity, produce cytotoxic molecules (H2O2, IL-1, TNF-α, NO, ROI) and stimulate lymphocyte responsiveness through presentation of TAA as well as production of immunostimulatory cytokines (e.g. IL-2). On the other hand, as pro-tumor effector, TAMs produce growth factors that promote cancer cell proliferation, dissemination, enhance angiogenesis, and suppress lymphocyte responsiveness via production both of immnosuppressive cytokines (e.g. IL-10) and prostanoïds.

In breast cancer, the increase of macrophages infiltration, and macrophage-mediated angiogenesis induced the high expression of MCP-1 (monocyte chemoattractant protein-1) and VEGF (vascular endothelial growth factor). Monocyte chemo-attractant protein-1 expressions in tumor cells was significantly correlated with the extent of TAM infiltration and both MCP-1 and VEGF expression have been positively correlated with TAM infiltration, angiogenesis and poor survival. Vascular endothelial growth factor promotes the proliferation, survival and migration of endothelial cells by binding to its receptors. The other factor responsible for increased macrophage infiltration in breast cancer is the macrophage colony-stimulating factor (CSF-1), which is important not only for macrophage recruitment but also for tumor vascularization and progression.

Tumor-associated macrophages also secrete proteases that degrade the extracellular matrix, for example a metalloproteinase (MMP)-9 that has emerged as an important modulator of angiogenesis and tumor development. Other proteases such as urokinase-type plasminogen activator and heparinase, release proangiogenic growth factors (e.g. FGF-β) that are sequestered by heparan sulphates proteoglycans in the extracellular matrix. But, MMP-9 may also have an antiangiogenic effect (at later stage) by processing the a3 chain of type IV collagen to the angiogenesis inhibitor. Tumor-associated macrophages also release thrombo-spondin-1, interferon-a and interferon-g which are antiangiogenic. Many cytokines (e.g. TGF-β, IL-1b, IL-6, TNF-α) are known to have pleiotropic effects, stimulating angiogenesis under