Analysis of cytokine producing profiles in local and systemic lymphocytes in sick building syndrome compared with ocular allergy

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Research Article

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Abstract

Purpose: We have previously studied clinical and allergological aspects of sick building syndrome (SBS) cases with ocular disorders and found that SBS is suggested to be partially induced by an allergic response. We analyzed the cytokine production profiles of conjunctival and peripheral blood lymphocytes in patients with SBS with ocular manifestations to further evaluate the pathophysiology of SBS from an immunological standpoint.

Methods: We obtained conjunctival samples and peripheral blood mononuclear cells (PBMC) from 15 cases of SBS with ocular findings and 49 cases of allergic conjunctival diseases (ACD) (allergic conjunctivitis (AC), atopic keratoconjunctivitis (AKC), and vernal keratoconjunctivitis (VKC)), and normal controls. Frequencies of cytokine-producing T cells were analyzed by flow cytometry based on an intracellular cytokine staining method.

Results: Although no significant difference was observed in the percentage of interferon (IFN)-g-producing CD4+ T cells in PBMC between patients with SBS and controls, the percentage of interleukin (IL)-4-producing PBMC CD4+ T cells in patients with SBS was significantly higher than that in controls. The percentage of IL-4-producing CD4+ T cells in the conjunctiva was significantly higher than that in controls, whereas it was significantly lower than those in AKC and VKC. A significant correlation was observed between the percentage of IL-4-producing CD4+ T cells in the conjunctiva and clinical score.

Conclusion: From these results it might be possible that SBS belongs to a kind of allergic disorder and IL-4 plays a role in the development of allergic disorders in SBS ocular lesions.

Introduction

Symptoms associated with poor quality of the indoor environment include various non-specific symptoms affecting the eyes, nose, throat, and skin and general symptoms like headache and tiredness, sometimes denoted as sick building syndrome (SBS) [1]. Irritation and dryness of the eyes together with a blocked or runny nose and dry throat are especially considered an important part of the oculonasal mucosal symptoms of SBS [2]. Symptoms of SBS occur only in specific buildings or dwellings and disappear when the person is out of the environment. We have previously studied clinical and allergological aspects of SBS cases with ocular disorders with special reference to allergic conjunctival diseases (ACD); allergic conjunctivitis (AC), atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC), from the standpoint of analyzing their similarities and differences, especially with respect to local immunological features, and reported that conjunctival lesions were observed in all cases and corneal abnormalities were found in two-thirds of cases of SBS [3]. Mean serum total IgE level in SBS was significantly higher than that in AC; however, it was significantly lower than those in AKC and VKC [3]. Eosinophil count in peripheral blood and the number of positive allergens in multiple allergen simultaneous test (MAST) were significantly lower in SBS than in AKC and VKC. Significant elevation of tear interleukin (IL)-4 was observed in SBS and ACD [3]. However, in contrast to ACD, elevation of other

cytokines in tears was not observed in SBS [3]. From these results, it is considered that SBS may be partially induced by an allergic response; however, the clinical and local immunological features of SBS suggest that SBS may belong to a different entity from ACD [3]. There remains a need for studies evaluating the pathophysiology of the ocular disorders found in SBS cases based on inflammatory cells on the ocular surface to highlight the responses observed in human cases. Therefore, our aim was to analyze the cytokine production profiles of conjunctival or peripheral blood lymphocytes in patients with SBS with ocular manifestations and evaluate the relationship of these parameters to clinical severity in cases of SBS with ocular complications in this study.

Materials And Methods

Study population

This was a consecutive case series study of 15 patients (4 male and 11 female) who attended Yokohama City University Medical Center with SBS symptoms, especially ocular irritation in a specific building. They were asked to complete a self-reporting questionnaire survey as previously proposed by Lu et al [4], and written informed consent was obtained from all patients. SBS symptoms were defined as one or more selected symptoms specified in the questionnaire for at least 1–3 days a week while at work in the office in the previous month, but which improved or disappeared after work or on days without work. The symptoms of SBS were identified individually as several groups including the eyes, as described previously [4]. Those with a past history of medical treatment for systemic diseases, such as hypertension, cardiac diseases, and hyperthyroidism, were excluded from the study population.

For comparative evaluation with SBS, 49 patients with ACD who also attended Yokohama City University Medical Center were included in this study. ACD was classified into AC, AKC, or VKC according to the guidelines for the diagnosis and treatment of conjunctivitis, and past reports [5–6]. The ACD patients consisted of 14 with AC (6 male and 8 female), 14 with AKC (6 male and 8 female), and 21 with VKC (17 male and 4 female). Thirteen normal (non-allergic) subjects (5 male and 8 female) were subjected to immunological testing for comparison.

Clinical grading

Clinical evaluation of ocular findings was carried out according to the ocular clinical grading system reported previously [6]. Ocular findings of slit lamp examination were recorded on the patients' first visit to our outpatient clinic. Ten objective ocular clinical findings of conjunctival, limbal, and corneal lesions were graded on a 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe; left and right eyes separately in each case). The total score of 10 findings, with a maximum of 30, taking the score of the more severe side in bilateral cases, was used as the clinical score.

Monoclonal antibodies

Phycoerythrin (PE)-conjugated anti-human interferon (IFN)-g monoclonal antibody (mAb) (mouse IgG1) and PE-conjugated anti-human IL-4 mAb (mouse IgG1) were purchased from Becton Dickinson (BD) Biosciences (San Diego, CA). PE-labeled isotype-matched immunoglobulin (Ig) (BD Biosciences) was used for the negative control. Fluorescein isothiocyanate (FITC)- or PE-Cy^{TM}5-conjugated anti-human CD3 mAb and PE-Cy^{TM}5 conjugated anti-human CD8 mAb were purchased from BD Biosciences. Fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse Ig was purchased from Dako (Glostrup, Denmark).

Cell preparation and culture

Conjunctival cell specimens from the upper palpebral conjunctiva were collected using a Cytobrush [7] (Cytobrush Small, Medscand, Malmo, Sweden) under topical anesthesia with xylocaine eye drops. These specimens were kept in 2 ml of Gibco Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific Inc., Göteborg, Sweden) on ice. These cells were then prepared for intracellular cytokine production assay according to the method described previously [8]. Six milliliters of heparinized venous blood was also obtained. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation (Nycomed Diagnostics, Oslo, Norway) and washed three times with RPMI-1640 medium. PBMC (4×10^6 cells/ml) were cultured in 24-well culture plates under the conditions described above.

Immunofluorescence staining and flow cytometric analysis

After stimulation with phorbol myristate acetate (PMA) and calcium ionophore, the cells were washed in phosphate-buffer saline (PBS) with 1% fetal calf serum (FCS) (staining buffer) and stained with FITC-conjugated anti-human CD3 mAb and PE-Cy^{TM}5-conjugated anti-human CD8 mAb for 30 minutes. The cells were washed twice with staining buffer and then fixed in cold PBS containing 2% paraformaldehyde for 15 minutes. After two washes in PBS, cells were washed in 0.1% saponin/PBS for permeabilization of the cell surface and resuspended in 100 µl of 0.1% saponin/PBS containing PE-conjugated anti-human IFN-g Ab (1 µg) or PE-conjugated anti-human IL-4 mAb (1 µg) for 30 minutes on ice. The cells were washed once in 0.1% saponin/PBS and once in staining buffer. The cells were resuspended in 500 µl staining buffer. Cells were analyzed on a FACScan™ flow-cytometer (BD Biosciences) using CELL Quest software (BD Biosciences). Negative isotype controls were used to verify the staining specificity of the antibodies used. Analysis gates were set on lymphocytes according to forward- and side-scatter properties. We analyzed frequency of cytokine-producing T cells of conjunctiva in the gates set on lymphocytes according to forward- and side-scatter properties. In this study we analyzed the frequencies of IFN-g- or IL-4-producing T cells in this gate as reported in our previous study [8]. CD3 and CD8 expression on T cells were investigated because CD4 expression disappeared after 24 h with PMA and calcium ionophore. We calculated the frequency of CD4 + T cells by (CD3 + T cell [%] - {CD3 + CD8 + T cell [%]}) [8].

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). One-way ANOVA with Tukey-Kramer test was conducted to identify differences among patient groups. Spearman's rank correlation coefficient was calculated to evaluate the correlation between cytokine levels and clinical scores. GraphPad Prism9J (MDF Co. Ltd., Tokyo, Japan) was used for statistical analysis. A P-value of < 0.05 was accepted as statistically significant.

Results

Percentage of IFN-g- or IL-4-producing CD4 + T cells in PBMC

The percentage of peripheral blood CD4 + T cells in ACD and SBS did not show a significant difference to that in healthy controls (data not shown). We compared the percentages of IFN-g- or IL-4-producing CD4 + T cells in PBMC between patients with SBS or ACD and healthy controls. The percentage of IFN-g-producing CD4 + T cells in PBMC did not show a significant difference among patients with SBS and ACD and normal controls except for between AC and AKC ($P < 0.05$) and between AC and VKC ($P < 0.05$) (Fig. 1). The percentage of IL-4-producing CD4 + T cells in PBMC from patients with SBS ($P < 0.01$), AKC ($P < 0.01$), or VKC ($P < 0.01$) was significantly higher than that in PBMC from normal controls (Fig. 2), whereas no significant difference was observed in the percentage of IL-4-producing CD4 + T cells among patients with SBS and different types of ACD (Fig. 2).

Percentage of IFN-g- or IL-4-producing CD4 + T cells in conjunctiva

The percentage of T cells detected in gated conjunctival lymphocytes was comparable among patients with SBS, ACD and normal controls, and was approximately 70% (data not shown). The percentages of conjunctival IFN-g-producing CD4 + T cells in patients with AKC ($P < 0.001$) and VKC ($P < 0.01$) were significantly higher than that in patients with SBS (Fig. 3). The percentages of conjunctival IFN-g-producing CD4 + T cells in patients with AKC ($P < 0.01$) and VKC ($P < 0.05$) were significantly higher than that in patients with AC (Fig. 3). The percentages of conjunctival IFN-g-producing CD4 + T cells in patients with AKC ($P < 0.001$) and VKC ($P < 0.01$) were significantly higher than that in normal controls (Fig. 3). There was no significant difference in the percentage of INF-g-producing CD4 + T cells between patients with VKC and AKC (Fig. 3). The percentage of conjunctival IL-4-producing CD4 + T cells in patients with SBS was significantly higher than that in normal controls ($P < 0.001$), whereas it was significantly lower than those in patients with AKC ($P < 0.01$) and VKC ($P < 0.0001$) (Fig. 4). The percentage of conjunctival IL-4-producing CD4 + T cells in patients with AC was significantly higher than that in normal controls ($P < 0.01$), whereas it was significantly lower than those in patients with AKC ($P < 0.001$) and VKC ($P < 0.0001$) (Fig. 4). No significant difference was observed in the percentage of conjunctival IL-4-producing CD4 + T cells between SBS and AC, and between AKC and VKC (Fig. 4).

Correlation between percentage of IFN-g- or IL-4-producing CD4 + T cells in PBMC or conjunctiva and clinical score in patients with SBS

The correlation coefficients between percentage of IFN-g- or IL-4-producing T cells in PBMC or conjunctiva and clinical score in patients with SBS are shown in Table 1. A significant correlation was observed only between the percentage of IL-4-producing T cells in the conjunctiva and clinical score (P = 0.0025) (Fig. 5). A relatively large coefficient was observed between PBMC IL-4-producing T cell percentage and clinical score (r = 0.48), but this did not reach statistical significance.

Table 1
Correlation between percentage of IFN-γ- or IL-4-producing CD4 + T cells in PBMC or conjunctiva and clinical score in patients with SBS

Cells	Correlation coefficient
IFN-γ-producing CD4 + T cells in PBMC	-0.056
IL-4-producing CD4 + T cells in PBMC	0.48 (P = 0.072)
IFN-γ-producing CD4 + T cells in conjunctiva	0.082
IL-4-producing CD4 + T cells in conjunctiva	0.72 (P = 0.0025)
PBMC, peripheral blood mononuclear cells; SBS, sick building syndrome; IFN-g, interferon-g; IL-4, interleukin-4.	

Discussion

A significantly elevated level of tear IL-4 detected by enzyme-linked immunosorbent assay (ELISA) has been reported in patients with SBS with ocular complications in our previous study [3]. IL-4 has been shown to induce the production of extracellular matrix components by fibroblasts [9], and the major source of IL-4 is thought to be type 2 helper T (Th2) cells. However, it is still unclear whether such increased cytokine production is caused by increased numbers of cytokine-producing cells or enhanced ability of cytokine production. Analysis of cytokine production has been approached by measuring each cytokine by ELISA or by measuring protein or mRNA levels. Flow cytometric analysis of cytokine-producing cells has been reported in several disorders including ocular allergic disease [8, 10–11]. Therefore, we applied this method to detect intracellular cytokines in individual cells and determined the percentage of IL-4- or IFN-g-producing CD4 + T cells in the conjunctiva and peripheral blood from patients with SBS in comparison with ACD. We found that the percentage of IFN-g-producing CD4 + T cells in

PBMC did not show a significant difference between patients with SBS and normal controls (Fig. 1); in contrast, the percentage of IL-4-producing CD4 + T cells in PBMC from patients with SBS was significantly higher than that in PBMC from normal controls (Fig. 2). These results indicate that the systemic cytokine production profile observed in CD4 + T cells of patients with SBS had different characteristics to that of normal controls and suggest that increased frequency of IL-4-producing CD4 + T cells may be one reason for increased type 2 cytokine production in patients with SBS. There have been few studies on the immunological features of SBS, whereas a previous study reported that eosinophil count in peripheral blood was a predictor of SBS [12]. This might support the systemic immunological finding of elevated percentage of IL-4-producing CD4 + T cells in PBMC. However, further evaluation is necessary regarding the systemic allergological aspects of SBS.

In this study, the significantly higher percentage of conjunctival IL-4-producing CD4 + T cells in patients with SBS compared with normal controls (Fig. 4) was similar to the finding of our previous report that significant elevation of tear IL-4 was observed in patients with SBS compared to that in normal controls [3]. The percentage of IFN-g-producing CD4 + T cells in the conjunctiva in SBS did not show a significant elevation compared with that in normal controls (Fig. 3). We reported the absence of a significant change in IFN-g level in tears of SBS patients compared with that in normal controls, and this seems to support our present results [3]. The result that the nasal lavage fluid level of IFN-g was significantly lower and the IL-4/IFN-g ratio was significantly higher in allergic than in control children led to the hypothesis that deficient release of type 1 helper (Th1) cytokines, such as IFN-g, plays an important role in the pathogenesis of allergic inflammation [13]. Regardless of whether defective IFN-g secretion is primary or a consequence of suppression by other cytokines, it would enhance the release of Th2 cytokines in allergic subjects, which in turn would facilitate the development of allergic inflammation, since SBS and AC, which are on the mild spectrum of ACD, showed a similar tendency regarding the percentage of IFN-g-producing CD4 + conjunctival T cells in this study (Fig. 3).

The significant elevation of the percentage of conjunctival IL-4-producing CD4 + T cells in SBS compared to normal controls ($P < 0.05$) may lead to the hypothesis that allergic reaction at least plays a partial role in the development of ocular disorder in SBS patients. However, the significantly lower percentage of conjunctival IL-4-producing CD4 + T cells in SBS compared to that in AKC or VKC might reflect the lower severity of allergic disorder or may suggest that SBS belongs to a different entity from ACD. The significantly lower percentage of conjunctival IFN-g-producing CD4 + T cells in patients with SBS than in those with AKC and VKC, with no significant elevation compared to normal controls (Fig. 3), showed similar results to IL-4-producing CD4 + T cells except for the comparison with controls (Fig. 4). However, the percentage of IFN-g-producing CD4 + T cells in PBMC showed significant differences among patients with ACD (Fig. 1), and this showed a significant opposite tendency to the frequency of IFN-g-producing CD4 + T cells in the conjunctiva among ACD; the reason for this discrepancy is unclear, but it might indicate that Th1 cells are only locally activated in the severe spectrum of ACD, such as AKC and VKC [8].

It has been revealed that the clinical severity of SBS correlated significantly with the percentage of IL-4-producing T cells in the conjunctiva, whereas the percentage of IFN-g-producing conjunctival T cells did

not correlate with clinical score in this study. This seems to be consistent with the tendency towards a switch from a predominantly type 2 response in the cytokine pattern that is observed in allergic disorders [14]; however, there have been few reports on the levels of IL-4 and IFN-g with regard to the clinical ocular severity of ACD. Leonardi et al. reported that IL-4 tear levels were increased in VKC and AKC compared with controls, but only IFN-g significantly correlated with corneal involvement [15]. They suggested that Th1 cells are locally activated and IFN-g has a role in the pro-inflammatory phase in the active phase of chronic allergic eye diseases, via IFN-g-secreting cells, such as conjunctival fibroblasts other than mononuclear cells [16–17]. As mentioned above, our present study carried out intracellular cytokine assays in CD4 + T cells in the conjunctiva and PBMC; therefore, this may explain the discordance between our results and those of Leonardi et al [15–17]. Thus, from the results that both type 1 and type 2 cytokines are produced in CD4 + T lymphocytes in SBS, with some difference in the percentage observed in the severe end of the spectrum of ACD, the possibility that IFN-g plays a role in the development of allergic disorders in SBS ocular lesions cannot be excluded, regardless of our results.

There are several limitations of this study as follows. Firstly, we have focused on the features of local cytokine production in SBS in comparison with ACD, and other biomarkers, such as chemokines, eosinophil cationic protein, matrix metalloproteinases, periostin etc., were not evaluated in this study. Multifactorial mechanisms should be evaluated in future studies. Secondly, this study was designed from the standpoint of ophthalmological aspects of SBS; therefore, the study population was small, and measurement of environmental parameters using calibrated instruments for each patient was not done because of the restriction of laboratory equipment and considerable diversity in the specific location of each patient. The causal elements might vary in each case, such as volatile organic compounds (VOC), odors, dust and bioaerosols. A VOC exposure study might be meaningful for direct clinical observation of ocular findings, but this type of experimental equipment is still restricted in Japan at present [18].

Declarations

Acknowledgments

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Author Contributions

All authors contributed to the study conception and design. Author contribution is as follows; Conceptualization: Ryosuke Izaki and Eiichi Uchio, Methodology: Kazuaki Kadonosono and Hiroaki Ozaki; Formal analysis and investigation: Ryosuke Izaki, Ayaka Kobayashi, Hideaki Fujita and Kazuhiro Harada; Writing - original draft preparation: Ryosuke Izaki; Writing - review and editing: Eiichi Uchio.

Funding interests

The authors have no relevant financial or non-financial interests to disclose.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Review Committee of Clinical Research Committee of Yokohama City University Medical Center.

Consent

Informed consent was obtained from all individual participants included in the study.

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Figures

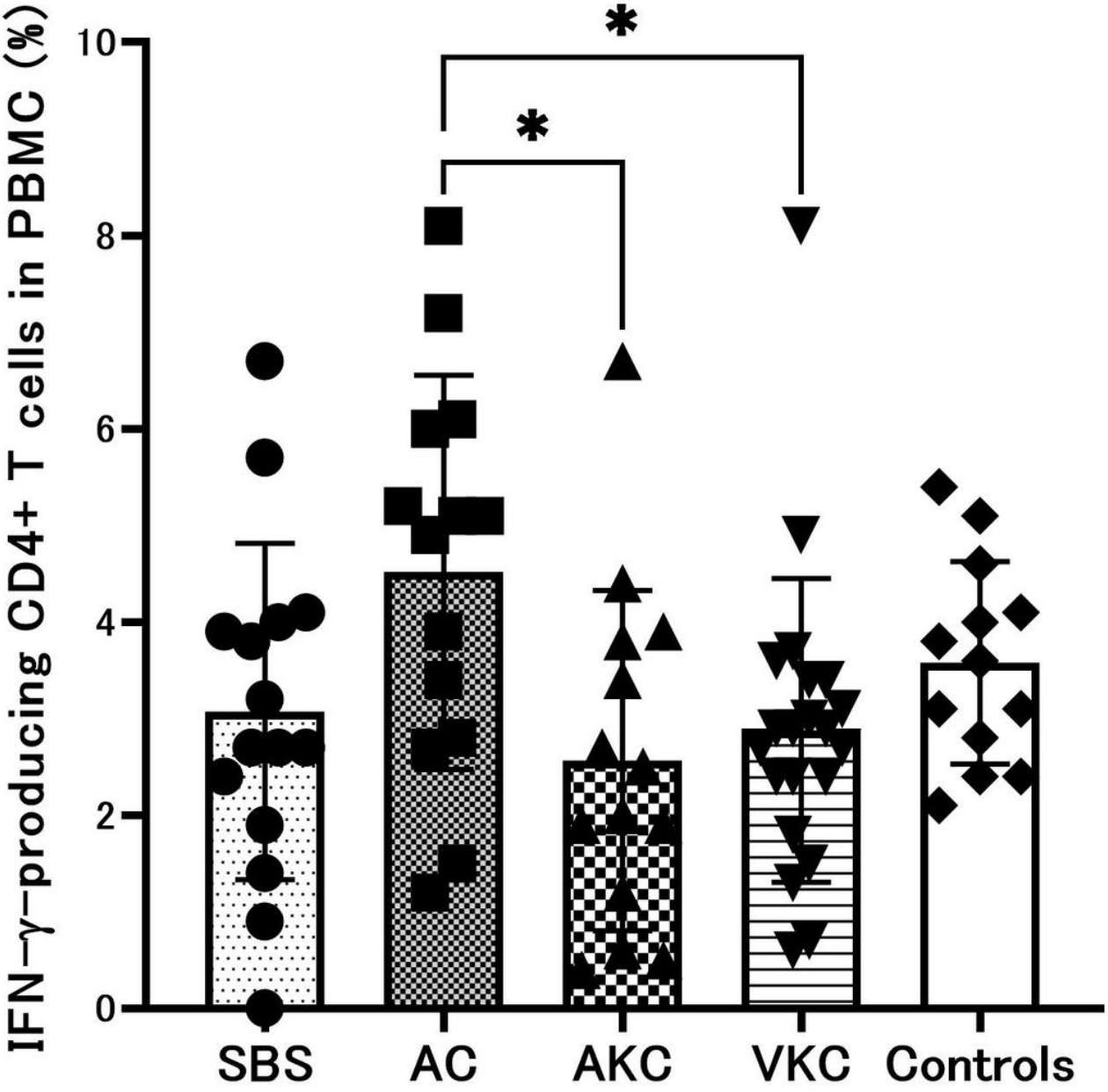

Figure 1

Percentage of IFN-g-producing CD4+ T cells in PBMC

The results are expressed in percent as mean ± SEM (*P < 0.05). PBMC, peripheral blood mononuclear cells; SBS, sick building syndrome; AC, allergic conjunctivitis; AKC, atopic keratoconjunctivitis; VKC, vernal keratoconjunctivitis.

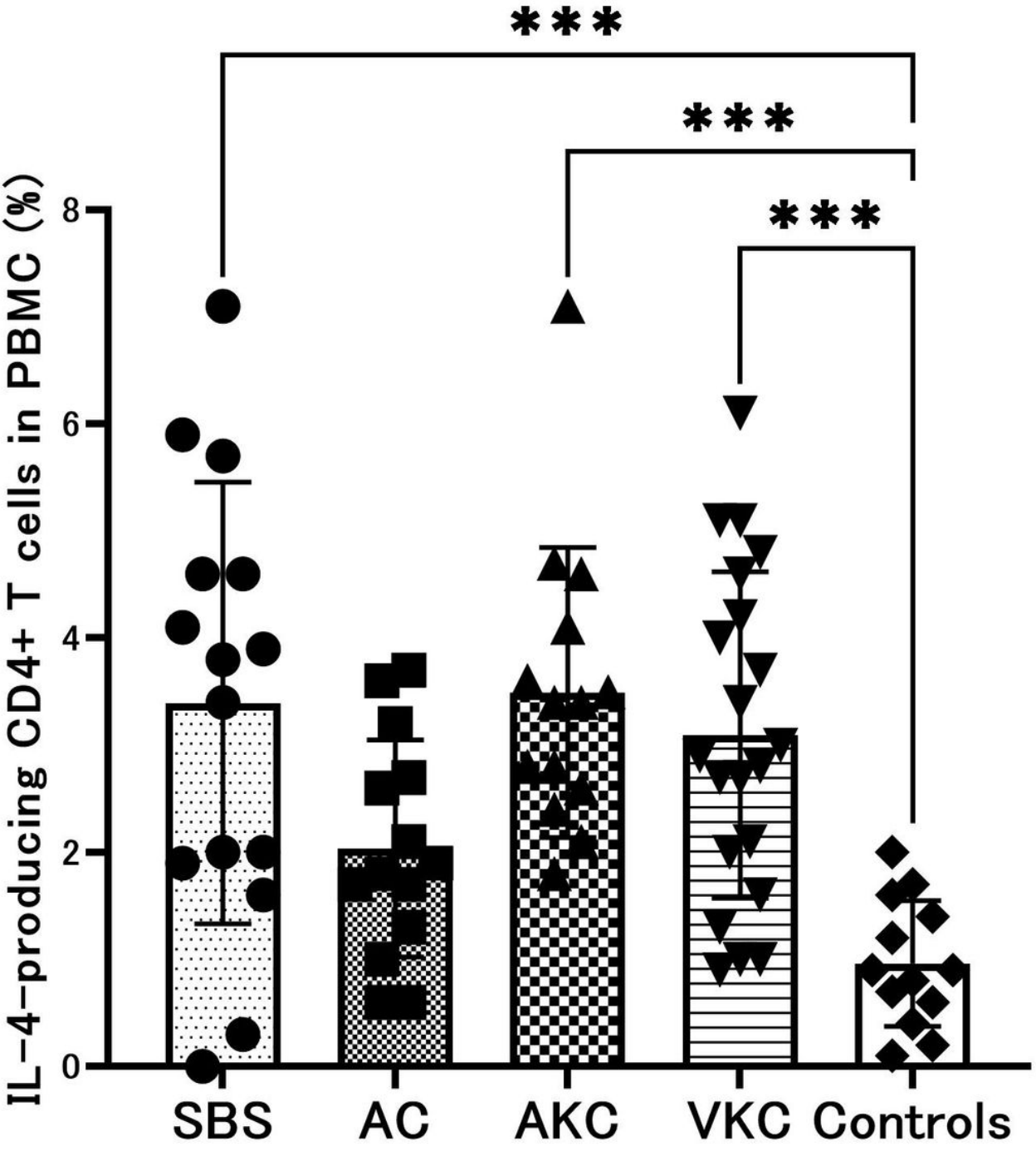

Figure 2

Percentage of IL-4-producing CD4+ T cells in PBMC

The results are expressed in percent as mean ± SEM (***P < 0.001).

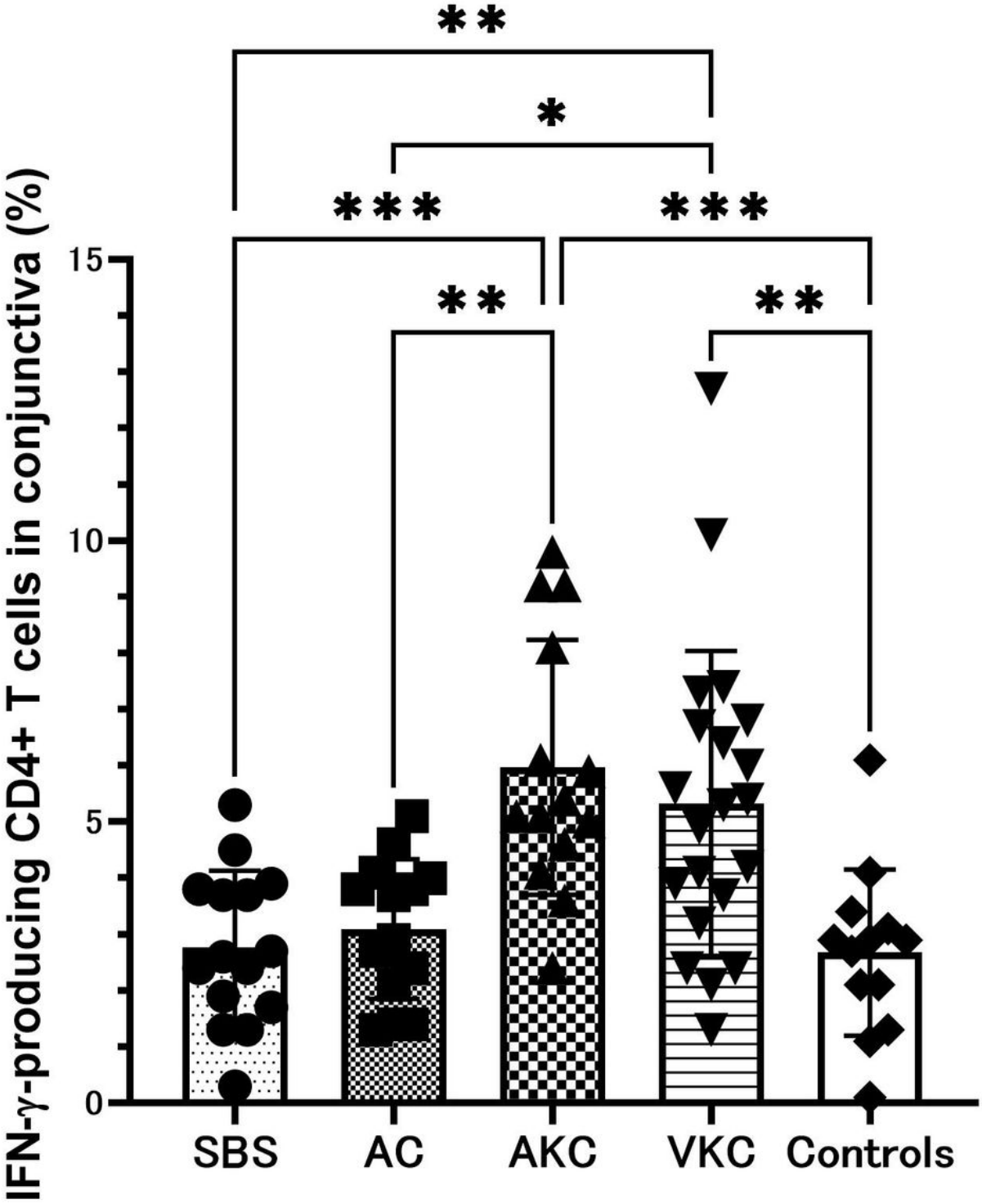

Figure 3

Percentage of IFN-g- producing CD4+ T cells in conjunctiva

The results are expressed in percent as mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

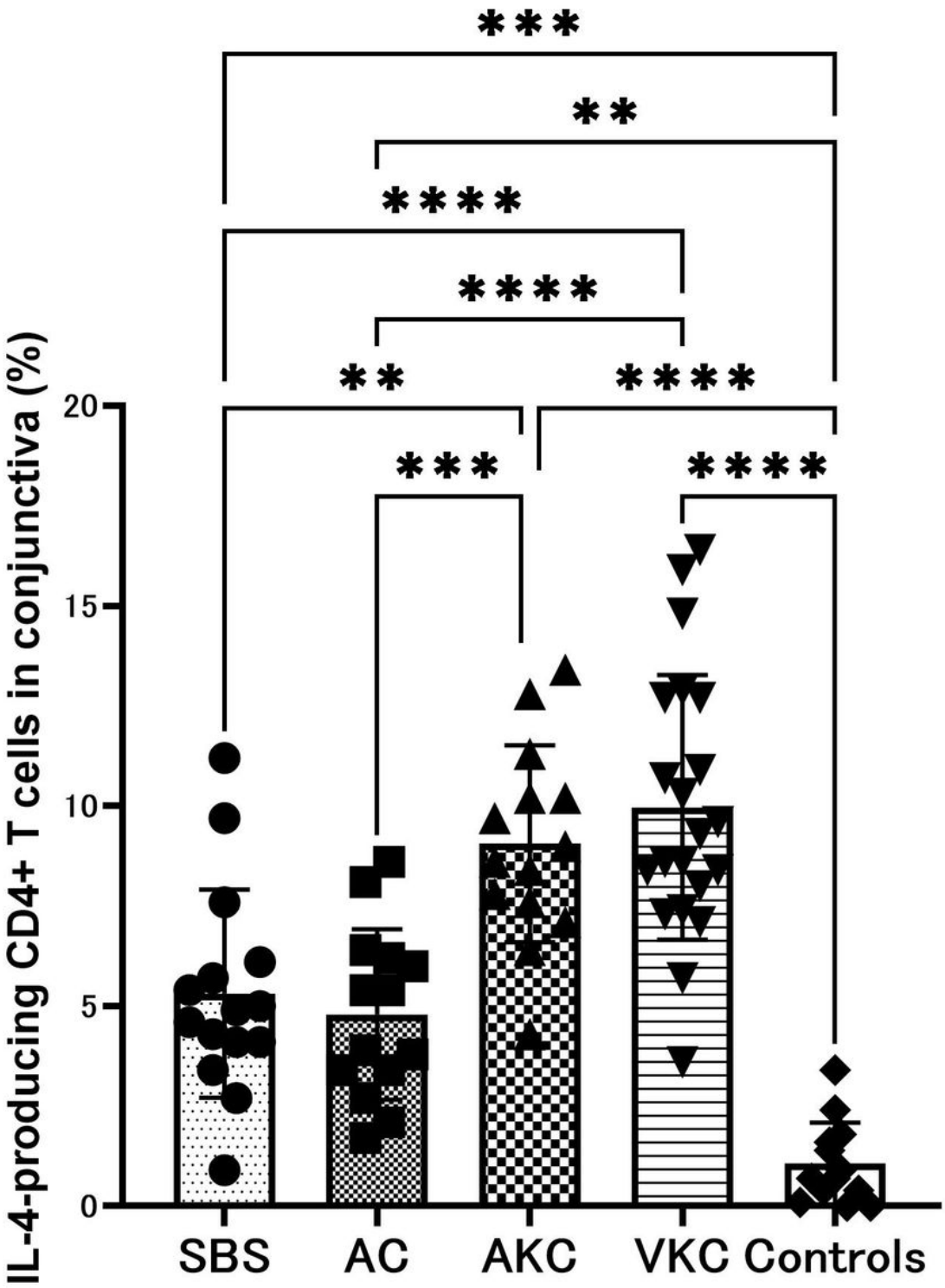

Figure 4

Percentage of IL-4-producing CD4+ T cells in conjunctiva

The results are expressed in percent as mean ± SEM (**P < 0.01; ***P < 0.001; ****P<0.0001).

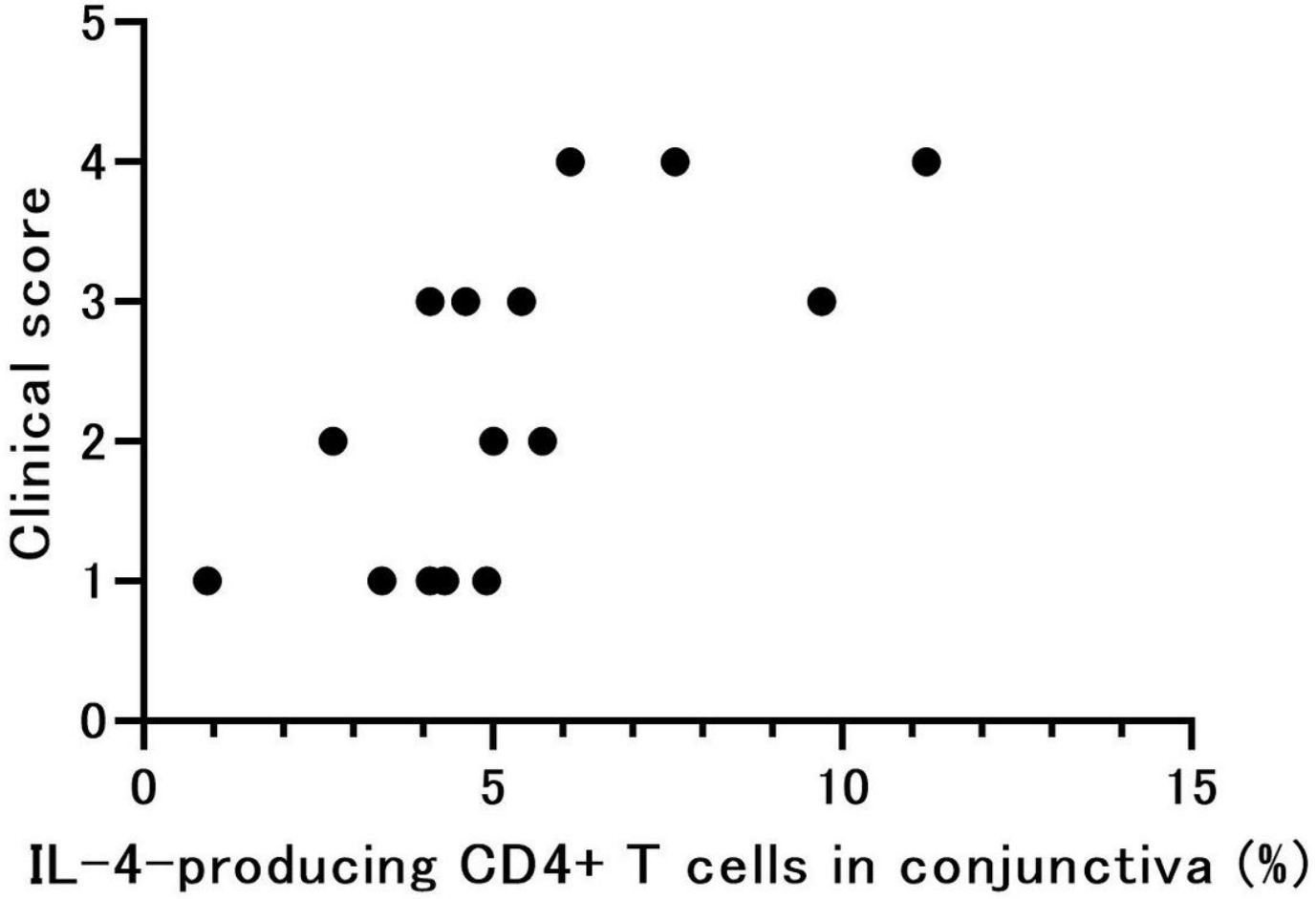

Figure 5

Correlation between percentage of IL-4-producing CD4+ T cells in conjunctiva and clinical score in patients with SBS

Correlation coefficient between percentage of IL-4-producing CD4+ T cells in conjunctiva and clinical score was significant (P = 0.0025, Spearman's rank test).