# The role of helper and regulatory T cells in the pathogenesis of vitiligo

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***Background:*** Alterations in cellular immunity, including $CD4^+$ T and $CD8^+$ T lymphocytes, have been proposed in the pathogenesis of vitiligo. There is also a proposed role for cytokines in the depigmentation observed in vitiligo. However, previous reports on the role of cytokines in the pathogenesis of vitiligo have been few in number.

***Objective:*** The purpose of this investigation was to assess the role of the major cytokines produced by T-helper 1 and 2 cells as well as T-helper 17 and regulatory T cells in the pathogenesis of vitiligo.

***Methods:*** Forty patients with vitiligo and 40 age- and sex-matched healthy control subjects were enrolled in the study. Serum interleukin (IL)-4, IL-6, IL-10, IL-17, interferon-$\gamma$, tumor necrosis factor-$\beta$, and transforming growth factor-$\beta$ levels were detected by enzyme-linked immunosorbent assay in both groups. The correlations of serum cytokine levels with age of onset, sex, duration of disease, type and activity of vitiligo, percentage of involved body area, Koebner positivity, family history, and the presence of associated autoimmune diseases were assessed.

***Results:*** Serum transforming growth factor-$\beta$ levels were significantly decreased in the vitiligo group compared with the control group ($P$ = .004). No difference was detected between the patient and control groups in mean levels of serum IL-6, IL-10, and tumor necrosis factor-$\beta$. In the patients with vitiligo, serum IL-17 levels were positively correlated with the extent of body area involvement (rho = 0.329, $P$ = .038).

***Limitations:*** Tissue cytokines compared with those in the peripheral blood were not measured.

***Conclusion:*** Although multiple factors have been implicated in the pathogenesis of vitiligo, reduced serum transforming growth factor-$\beta$ levels, as observed in patients in the current investigation, may contribute to enhanced cellular immunity. This may facilitate the occurrence of vitiligo by leading to diminished maturation of regulatory T cells, followed by impaired inhibition of inflammation. (J Am Acad Dermatol 2009;60:256-60.)

Vitiligo is an idiopathic depigmentary skin disorder characterized by selective destruction of melanocytes. Recent observations support the role of altered cellular immunity, autoimmunity, and a role for cytokines in the pathogenesis of vitiligo.[1]

*Abbreviations used:*

| | |
|---|---|
| ELISA: | enzyme-linked immunosorbent assay |
| IFN: | interferon |
| IL: | interleukin |
| TGF: | transforming growth factor |
| Th: | T helper |
| TNF: | tumor necrosis factor |
| Treg: | regulatory T cell |

Naive $CD4^+$ helper T cells are known to develop into 4 types, namely T-helper 1 (Th1), Th2, Th17, and regulatory T cells (Tregs). Th1 cells primarily produce interferon (IFN)-$\gamma$ and tumor necrosis factor (TNF)-$\beta$; Th2 cells synthesize interleukin (IL)-4, IL-5, and IL-13; Th17 cells produce IL-17 and IL-6; and Tregs synthesize IL-10 and transforming growth factor (TGF)-$\beta$.[2,3] A novel hypothesis has been suggested that skewing of responses toward Th17 or Th1 and away from Tregs and Th2 cells may be

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responsible for the development and progression of autoimmune diseases.[4]

Previous studies of peripheral blood cytokine derangements in vitiligo have been incomplete and have reported different results. To our knowledge, although peripheral blood cytokines produced by Th1 cells in vitiligo have been studied, the role of Th2 cells, Th17 cells, and Tregs have not been investigated. The aim of this study was to clarify cellular immune function in the pathogenesis of vitiligo by determining serum cytokines as indicators of helper and Treg functions.

## METHODS

### Participants

Forty patients with vitiligo and 40 age- and sex-matched healthy control subjects were included in the study. The patient group included 21 women (52.5%) and the control group included 17 women (42.5%). The mean ages of patients and control subjects were 39.8 ± 16.5 and 33.9 ± 12.5 years, respectively. Age, sex, duration of disease, age of onset, family history, any systemic or autoimmune diseases, and Koebner phenomenon positivity were noted. Type of vitiligo (localized, generalized, segmental, acral/acrofacial) and involvement of body area in quartile percentiles were recorded. The course of vitiligo was defined as stable when new lesions had not appeared within 1 year. Patients had not used any medications in the preceding 4 weeks.

Complete blood cell count, fasting blood sugar and thyroid function tests, antithyroglobulin antibodies, and vitamin B12 and folate levels were detected. All patients and control subjects provided signed informed consent after the study was explained.

### Clinical characteristics of patients

The duration of vitiligo was 10.6 ± 10.1 years. Family history of vitiligo was present in 9 patients (22.5%). Eight patients (20%) had other autoimmune disorders such as type 1 diabetes mellitus, autoimmune thyroid diseases, alopecia areata, or a combination of these. In all, 24 (60%) of the patients were in active period, although Koebner phenomenon was positive in only two patients (5%). Generalized type vitiligo was detected in 16 (40%) patients whereas localized and acral/acrofacial forms were detected in 13 (32.5%) and 11 (27.5%) of the patients, respectively. Involvement of the body area was under 25% in 36 patients (90%), between 26% and 50% in two, between 51% and 75% in one, and more than 75% in one.

### Methods

Peripheral venous blood samples were drawn from patients and control subjects, and sera were obtained and stored at −80°C until analysis.

**Table I.** Comparison of mean ± SD serum cytokine levels in vitiligo and control groups

| | Vitiligo group (n = 40) | Control group (n = 40) | *P* |
|---|---|---|---|
| IL-6, pg/mL | 7.9 ± 0.7 | 8.3 ± 1.9 | .188 |
| IL-10, pg/mL | 8.1 ± 6.5 | 6.6 ± 5.4 | .269 |
| TNF-β, pg/mL | 39.2 ± 155.9 | 19.5 ± 19.6 | .432 |
| TGF-β, pg/mL | 1043.4 ± 2460.5 | 7078.8 ± 12134.9 | .004* |

*IL*, Interleukin; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor.
*Independent samples *t* test, $P < .05$.

Serum IL-4, IL-6, IL-10, IL-17, IFN-γ, TGF-β1 (BioSource Int, Camarillo, CA), and TNF-β (Bender Medsystem, Vienna, Austria) levels were detected quantitatively by the enzyme-linked immunosorbent assay (ELISA) method in both groups. ELISA tests were studied according to manufacturer instructions.

The correlation of serum cytokine levels with age of onset, sex, duration of disease, type and activity of vitiligo, percentage of involved body area, Koebner positivity, family history, and the presence of any autoimmune diseases were assessed.

### Statistical analysis

Differences in means of the continuous variables between groups were compared by means of the independent samples *t* test. When the groups were smaller than 30 subjects, Mann-Whitney U or Kruskal-Wallis test (if more than two groups were compared) was used. Pearson and Spearman correlation tests were used for the relation between quantitative parameters. The significance level was set at *P* less than .05. Statistical analyses were performed using a software package (SPSS, Version 15.0 for Windows, SPSS Inc, Chicago, IL).

## RESULTS

Serum TGF-β levels were significantly decreased in patients with vitiligo compared with control subjects (independent samples *t* test, $P = .004$) (Table I). Statistical significance was detected in generalized, acral/acrofacial, and localized forms of vitiligo, when each was compared with control subjects (Mann-Whitney U, respectively: $P = .006$, $P = .007$, and $P = .015$) (Table II). However, there was no difference in means of serum IL-6, IL-10, and TNF-β levels between the patient and control groups (Table I). Serum IL-10 levels were not statistically different among the clinical types of vitiligo when each was compared with that of control subjects (Table II). In both groups, IL-4 and IFN-γ were found in serum at minimal levels that were out of measurable range.