Expression of Transforming Growth Factor Beta 1 in Vitiligo

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Abstract

Background: Vitiligo is characterized by selective destruction of melanocytes. Transforming growth factor beta 1 (TGF-β1) is the main product of regulatory T cells (Tregs) which are deficient in vitiligo. Thus, lower levels of serum TGF-β1 are expected in vitiligo. However, TGF-β1 is a keratinocyte-derived cytokine with inhibitory effect on melanocyte activity. Thus it may be expected to be higher in the sera of patients with vitiligo.

Objectives: To evaluate the serum level of TGF-β1 in vitiligo and its correlation with disease parameters, in order to determine its actual role in the pathogenesis of vitiligo.

Patients and Methods: Serum levels of 24 patients with vitiligo and 23 controls were collected for quantification of TGF-β1 by enzyme-linked immunosorbent assay kit.

Results: There was no significant difference between mean serum TGF-β1 in patients and controls. However, mean serum TGF-β1 was higher in patients with active disease than in patients with inactive disease and this difference was statistically significant (p=0.024). No correlation was detected between mean serum levels of TGF-β1 and other disease parameters.

Conclusions: The results of this study demonstrate that serum TGF-β1 does not seem to have direct role in the pathogenesis of vitiligo. However, this does not exclude its cutaneously produced effect on production of vitiligenous lesions.

Key Words: Vitiligo – Transforming growth factor beta 1 – Regulatory T cells – Keratinocytes.

Introduction

VITILIGO is an idiopathic depigmentary skin disorder characterized by selective destruction of melanocytes. Alterations in cellular immunity, including CD4+ T and CD8+ T lymphocytes have been proposed in the pathogenesis of vitiligo [1].

In the epidermis, the epidermal melanin unit consists of the close interaction of a melanocyte and an associated pool of keratinocytes and several keratinocyte-derived cytokines that affect melanocyte migration, proliferation and differentiation, so that the epidermal microenvironment, made of a very prevalent number of keratinocytes may be considered a crucial milieu for the normal life and functions of epidermal melanocytes [2].

Though previous reports on the role of cytokines in the pathogenesis of vitiligo have been few in number, it seems that many cytokines are involved in the depigmentation observed in vitiligo, among which is Transforming growth factor beta 1 (TGF-β1) [3]. TGF-β1 is produced by many cell types including epidermal keratinocytes [4]. It is localized in the upper differentiated layers of the epidermis and it has an antiproliferative effect on many cell types including epidermal keratinocytes [4]. It is localized in the upper differentiated layers of the epidermis and it has an antiproliferative effect on many cell types including epidermal keratinocytes [5]. TGF-β1 does not only have an inhibitory effect on keratinocytes, but acts on melanocytes via specific receptors producing paracrine inhibition of human melanocyte proliferation and melanogenesis [6].

TGF-β1 is the main product of regulatory T cells (Tregs), which are defined as CD4+ T cells in charge of suppressing potentially deleterious activities of T helper (Th) cells. It was proposed that suppressor T cells would be capable of inhibiting other T cells and thereby mediate immunological tolerance and self/non-self discrimination [7].

Transforming growth factor beta 1 has been suggested to be an important mediator of Treg-mediated suppression [8]. TGF-β1 can suppress T cell responses either through a direct or an indirect pathway. For instance, TGF-β1 can directly exert its antiproliferative effects on CD4+ T cells due to its ability to inhibit IL-2 production and to upregulate cell cycle inhibitors. TGF-β1 can also directly inhibit the differentiation of Th1 and Th2 cells via a downregulation of the transcription factors T-bet and GATA-3 that are required for the expression
of IFN-γ and IL-4, respectively. In addition, TGF-β1 can inhibit the activation of macrophages and their ability to produce pro-inflammatory cytokines as well as preventing the maturation of dendritic cells (DCs) and decrease MHC-class II expression by DCs and subsequently decreases the ability of DCs to present antigens to T cells. While TGF-β1 suppresses Th1 and Th2 differentiation, conversely, it also induces the differentiation and development of Tregs [9].

Since vitiligo is an autoimmune disease affecting melanocytes, with a role for Tregs in its pathogenesis and since TGF-β1 inhibits melanocytes and regulates Tregs it is expected to have a role in vitiligo.

Subjects and Methods

Patients:

Twenty-four patients with vitiligo and twenty-three age- and sex-matched healthy control subjects were included in the study. The mean ages of patients and control subjects were 39.38 ± 18.726 and 36.30 ± 11.467 years, respectively. The patients group included 9 women (37.5%) and the controls group included 13 women (56.5%). The 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, acral/acrofacial) and involvement of body area were recorded. Vitiligo was classified as stable when no new lesions developed for 1 year. Patients had not used any medications in the preceding 4 weeks. All patients and control subjects provided signed informed consent after the study was explained.

Clinical characteristics of patients:

The mean disease duration was 12.2 ± 6.456 years. Family history was positive in 8 patients. Mean age of onset was 25.25 ± 15.927 years. Koebner phenomenon was positive in 5 patients. One patient had associated other autoimmune disease which was juvenile onset diabetes mellitus. Nineteen patients had generalized disease and 5 patients had localized/acrofacial disease. The mean extent of the disease was 45% ± 23.73%. Half of the patients (50%) had evidence of active disease.

Methods:

Peripheral venous blood samples were drawn from patients and control subjects and sera were obtained and stored at −80°C until analysis. Serum TGF-β1 (Biosource Int, Camarillo, CA) levels were detected quantitatively by the enzyme-linked immunosorbent assay (ELISA) method. ELISA test was studied according to manufacturer instructions. The correlation of serum cytokine levels with patient’s age, 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 disease were assessed.

Statistical analysis:

Data were statistically described in terms of range, mean ± standard deviation (± SD), median, frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the studied groups was done using Mann Whitney U independent samples. For comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Spearman rank correlation equation for non normal variables. Multivariate analysis models were used to test for the preferential effect of the independent variable(s) on the dependent variable(s). A probability value (p=value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft windows.

Results

The study included 24 patients and 23 healthy controls. The relevant clinical data are shown in Table (1).

<table>
<thead>
<tr>
<th>Clinical characteristics of patients</th>
<th>Cases (n=24)</th>
<th>Controls (n=23)</th>
<th>(p=value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.38±</td>
<td>36.30±</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td>18.726</td>
<td>11.467</td>
<td></td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>(15/9)</td>
<td>(10/13)</td>
<td>0.248</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>12.2±6.446</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent percentage</td>
<td>45±23.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease activity ratio</td>
<td>(12/12)</td>
<td></td>
<td></td>
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<tr>
<td>Type of vitiligo</td>
<td>(19/5)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(Generalised/localised)</td>
<td></td>
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In the present study, the serum level of TGF-β1 in patients was 19.458±8684 Pg/ml and in controls was 22.276±515 Pg/ml. There was no significant difference between serum TGF-β1 in patients and controls (p=0.302).

No significant difference in serum TGF-β1 among patients as regards age, sex, age of onset,
duration, type of vitiligo, percentage of involved body area, Koebner positivity, family history and the presence of any autoimmune disease. However, serum TGF-β1 was higher in patients with active disease than in patients with inactive disease and this difference was statistically significant (p = 0.024). Serum levels of TGF-β1 were higher in controls than in patients with inactive disease and the difference was statistically significant (p = 0.022).

However, serum levels of TGF-β1 were higher in patients with active disease than in controls with no statistical significance (p=0.54).

Tables (2,3) show patients and controls levels of TGF-β1 and demonstrate relation between active lesions and TGF-β1, respectively.

<table>
<thead>
<tr>
<th>Table (2): Serum levels of TGF-β1 in patients and controls.</th>
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<tbody>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>TGF-β1 (Pg/ml)</td>
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</table>

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<tr>
<th>Table (3): Comparison of serum levels of TGF-β1 in patients with active disease and those with inactive disease.</th>
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<tbody>
<tr>
<td>Cases with active disease</td>
</tr>
<tr>
<td>TGF-β1 (Pg/ml)</td>
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</table>

Discussion

Previous reports concerning cytokine expression in patients with vitiligo have been limited and the results contradictory. There is growing evidence that cytokines that are important in the pathogenesis of autoimmunity may play a role in the depigmentation associated with vitiligo [10]. Moretti et al. [11] investigated the level of TGF-β1 in lesional, perilesional skin of patients as well as normal skin of controls. Decreased TGF-β1 expression was found in all samples. They suggested that the decreased expression of TGF-β1 is due to the fact that this cytokine is produced by keratinocytes, which are possibly impaired in vitiligo. Later, Basak et al. [3] investigated the level of TGF-β1 in sera of patients with vitiligo as well as of controls. They found that serum TGF-β1 was significantly decreased in patients with vitiligo compared to controls. They suggested that decreased TGF-β1 levels in vitiligo may result in a dominant CD8+ T lymphocytes response, diminished maturation of Tregs and impaired inhibition of inflammation, thus facilitating the occurrence of vitiligo.

A novel hypothesis has been suggested that skewing of responses toward Th17 or Th1 and away from Tregs and Th2 cells may be responsible for the development and progression of autoimmune disease [12]. T-helper 1 (Th1), Th2, Th17 and Tregs are all the subtypes of CD4 helper cells. Th1 cells primarily produce interferon (IFN)-γ and tumor necrosis factor (TNF)-β; 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 TGF-β1. TGF-β1 is the main product of Tregs, which have several immunomodulatory effects such as anti-inflammatory role to maintain tolerance to self-antigens. In addition, Tregs have the ability to inhibit the responses of CD8+ T cells, natural killer cells and CD4+ T cells so that Tregs may be important in the prevention of autoimmune disease. Therefore, autoimmune diseases may exacerbate in the absence of Tregs and thus it would not be surprising to expect lower levels of Tregs and hence TGF-β1 in patients with vitiligo [13].

Which explain the lower serum levels of TGF-β1 in vitiligo patients than that of controls detected in our study, however this difference was statistically insignificant (p=0.302).

Nevertheless, serum levels of TGF-β1 were found to be elevated in patients with active disease than those with stable disease in a statistically significant manner (p=0.024).

Serum levels of TGF-β1 were also higher in patients with active disease than in controls in a statistically non significant manner (p=0.54). Serum levels of TGF-β1 were higher in controls than in patients with inactive disease in a statistically significant manner (p=0.022). Therefore, we suggest that the inflammatory response that occurs early in vitiligo is associated with an early increase in serum levels of TGF-β1. This elevation eventually settles down in stable lesions to a degree that is less than normal.

Thus it seems that in initial stages of the disease, there is a brief surge of serum TGF-β1, caused by recruitment of inflammatory cells, leading to transient elevation in its levels. Later on, this process becomes inefficient or abnormal and incapable of suppressing the inflammatory response as the cells shift towards Th1 and Th17 and away from Th2 and Tregs. Therefore, TGF-β1 levels start to decrease. TGF-β1 is important for the differentiation and development of Tregs [9] and it is their main product so a vicious circle is produced where TGF-β1 levels decrease till it eventually settles down in stable lesions to less than its normal levels.

Conclusion:

The results of this study demonstrate that serum TGF-β1 does not seem to have a direct role in the
pathogenesis of vitiligo. However, this does not exclude its cutaneously produced effect on melanocytes via specific receptors producing inhibition of their proliferation and melanogenesis. Therefore, further studies combining both tissue cytokines in addition to peripheral blood will be the key to clarifying the role of TGF-β1 in the pathogenesis of vitiligo.

References