CD4⁺CD25⁺ and CD4⁺CD25<sup>high</sup> regulatory T cells in disseminated and localized forms of allergic contact dermatitis: relation to specific cytokines

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Abstract: The aim of this study was to evaluate regulatory T lymphocytes (Tregs) in the course of allergic contact dermatitis (ACD) and to elucidate the role of IL-10 and TGF-β in Tregs activity. Peripheral blood CD4⁺CD25⁺ and CD4⁺CD25<sup>high</sup> cells were determined by flow cytometry in patients with acute disseminated ACD (‘ad’, n = 36), acute localized ACD (‘al’, n = 26), and disseminated ACD during remission (‘rd’, n = 27) as well as in controls (n = 22). Serum levels of cytokines were measured using ELISA. The mean percentage of CD4⁺CD25⁺ and CD4⁺CD25<sup>high</sup> cells in patients with ad ACD was significantly higher than in controls (p < 0.01) and the remaining patients (p < 0.05). Both cell populations were significantly elevated in persons with widespread skin lesions (p < 0.05). In ad patients the CD4⁺CD25⁺ increased during three weeks of disease, although the significant increase of CD4⁺CD25<sup>high</sup> was noted only in the third week. Patients with ad ACD showed a significantly decreased serum level of TGF-β1 as compared with controls and the remaining ACD patients. IL-10 level did not differ between all groups. The elevated population of CD4⁺CD25<sup>high</sup> cells in ad ACD patients, and its dependence on the extension of skin lesions, suggest a role of Tregs in regulating the course of ACD. The growing Tregs percentages may indicate their peripheral generation during ACD. The development of lesions despite an increased population of Tregs suggests their functional defect. The role of TGF-β1 in the suppressive activity of Tregs cannot be excluded. (Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 2, pp. 255–262)

Key words: allergic contact dermatitis, clinical course, regulation, regulatory T lymphocytes

Introduction

Allergic contact dermatitis (ACD) is an antigen-specific, T cell-dependent inflammatory skin disease, which develops as a result of contact with hapten in allergic persons. The allergic reaction occurs in two phases: sensitization and elicitation. The Langerhans’ cells (LC) play a key role in the development of ACD: they process the allergen and present it to T cells in draining lymph nodes (DLN). The migration and maturation of LC are initiated and regulated by cytokines in epidermis: tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, IL-12 and granulocyte macrophage-colony stimulating factor (GM-CSF) [1–3]. In the elicitation (effector) phase of ACD, specific T cells, both CD4⁺ and CD8⁺, proliferate and when activated, secrete inflammatory cytokines (IL-2, IFN-γ) which also activate keratinocytes. Keratinocytes express intracellular cell adhesion molecule-1 (ICAM-1), which allows for the cells to interact with T lymphocytes. These cells also express molecule HLA-DR, which allows the presentation of antigen to T lymphocytes. Activated keratinocytes produce cytokines: IL-1, IL-6, GM-CSF, which recruit and activate T cells.
The clinical effect of the contact hypersensitivity reaction is an inflammatory process in the skin. Most frequently, skin lesions are limited to the area of contact with the allergen (stage I) or spread to the nearest areas (stage II). In rare cases, lesions are disseminated or generalized (stage III) [4–6].

Regulatory T lymphocytes (Tregs) are a subset of T cells population with immunosuppressive activity. It has been shown that Tregs have a role in the regulation of the Th1 and Th2 activity, a role in defending against infections and neoplasms, and in maintaining immunological self-tolerance [7–11].

To date, two main types of Tregs have been described: natural Tregs, and induced or adaptive Tregs. Natural Tregs are physiologically produced by the thymus; they constitutively express the interleukin (IL)-2 receptor α chain (CD25), [12]. The marker of the natural Tregs is the transcription factor forkhead box P3 (FOXP3); its expression is necessary for the development and suppressive function of the cells [13–15]. Adaptive Tregs are induced from native T cells by a specific mode of antigen stimulation, especially in a particular cytokine milieu [16, 17]. They include Tregs which secrete IL-10 (T r1), cells secreting transforming growth factor (TGF)-β T helper (Th)3, γ/δ T cell Receptor (TCR)-expressing CD4+CD8− T cells and CD8+CD28− T cells [15, 18]. Lymphocytes CD4+CD25+ may have different degrees of CD25 expression. The population of Tregs FoxP3+ cells is identified as CD4+ cells with a high degree of CD25 expression (CD4+CD25high) [14].

The role of Tregs in allergic contact dermatitis has been studied in experimental conditions in vitro or in animal models, but in humans it has been evaluated mainly on patch test reactions [19–23]. This is why the examinations concern only the induction phase or the very early stage of effector phase of ACD and very limited (only one small skin lesion) form of disease.

The aim of this study was to evaluate the role of the regulatory T lymphocytes (Tregs) in allergic contact dermatitis (ACD) and the relationships between Tregs and the clinical course of disease, as well as to elucidate the significance of IL-10 and TGF-β in the suppressive Tregs activity.

Material and methods

Three groups of patients with allergic contact dermatitis (n = 89) and a group of healthy persons as a control group (n = 22) were enrolled in this study. The first group of ACD patients (group I, n = 36) consisted of persons with acute disseminated form of eczema (ad). The second group of patients comprised those with disseminated skin lesions during remission (group II, n = 27, rd). The third group included patients with acute eczema limited to one lesion (group III, n = 26, al). The diagnosis of ACD was established in each patient on the basis of thoroughly taken history of disease before and after patch testing and clinical examination. The extent and severity of skin lesions were calculated using the eczema area and severity index (EASI) scoring system for assessing the activity of AD, elaborated by Hanifin [24], adapted by authors for assessing severity of skin lesions in ACD and described in detail elsewhere [25]. None of the patients included in this study had clinical symptoms or laboratory signs of skin bacterial infection, nor had they been taking corticosteroids and any other immunotopic medication for at least four weeks.

The analysis of peripheral blood leukocytes was made using a hematological analyzer (MAXM, Beckman Coulter). The value of leukocytes was presented in G/L, and lymphocytes presented in percentages (%) and in absolute values (G/L). Subpopulations of peripheral blood lymphocytes were calculated using flow cytometry (Coulter Cytomics FC 500). The surface markers on lymphocytes were detected by incubation with the appropriate monoclonal antibodies (mAbs): anti-CD3 (phycoerythrin-cyanin 5, PECy5 conjugated), anti-CD4 (phycoerythrin-cyanin, PECy conjugated), anti-CD25 (phycoerthin Red, ECD conjugated), (Dako, Denmark). Serum levels of cytokines (IL-10 and TGF-β) were measured using ELISA with the commercially available enzyme-linked immunosorbent assay kit (Quantikine, R&D Systems, Minneapolis, MN, USA).

Statistical analysis. Results were presented as a mean of the absolute values and as a mean of the percentage of CD4+CD25+ and CD4+CD25high and SD, calculated on the basis of leukogram. Data was compared between all groups of ACD patients and healthy persons using two-tail, non paired t-test. Pearson’s correlation test was used to assess the correlation between the percentage of peripheral blood CD4+CD25+ and CD4+CD25high cells. A p value of less than 0.05 was regarded as significant.

Results

Eighty nine patients (42 women and 47 men, mean age 46.1 ± 14.6 years, range 20–84) with allergic contact dermatitis, and 22 healthy persons as a control (13 women and nine men, mean age 41.4 ± 15.6 years, range 23–82) participated in this study. The characteristics of the examined and control groups are presented in Table 1.

The mean percentage of CD4+CD25+ and CD4+CD25high cells in patients with acute disseminated lesions was significantly higher than in the control group (p < 0.01) and in the remaining ACD patients (p < 0.05), (Figures 1 and 2). There was a pos-
Table 1. Characteristics of allergic contact dermatitis patients and control group

<table>
<thead>
<tr>
<th></th>
<th>Patients with disseminated form of ACD</th>
<th>Patients with localized ACD (al)</th>
<th>Control (c)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Acute (ad)</td>
<td>Remission (dr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>26</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Sex (f/m)</td>
<td>13/23</td>
<td>12/14</td>
<td>17/10</td>
<td>13/9</td>
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<tr>
<td>Age (years)</td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>48.8 ± 15.5</td>
<td>47.9 ± 16.3</td>
<td>42.4 ± 12.0</td>
<td>41.4 ± 15.6</td>
</tr>
<tr>
<td>Range</td>
<td>20–84</td>
<td>20–82</td>
<td>21–65</td>
<td>23–82</td>
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<tr>
<td>Duration of disease</td>
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<tr>
<td>(years)</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>7.3 ± 9.1</td>
<td>5.2 ± 5.9</td>
<td>6.4 ± 5.6</td>
<td>–</td>
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<tr>
<td>Range</td>
<td>1 month – 30 years</td>
<td>3 months – 20 years</td>
<td>6 months – 21 years</td>
<td>–</td>
</tr>
<tr>
<td>Duration of present</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>lesions (weeks)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>2.97 ± 1.7</td>
<td>–</td>
<td>2.85 ± 1.4</td>
<td>–</td>
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<tr>
<td>Range</td>
<td>3 days – 8 weeks</td>
<td>–</td>
<td>1–7 weeks</td>
<td>–</td>
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<tr>
<td>EASI* score</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>26.3 ± 15.3</td>
<td>19.5 ± 13.9</td>
<td>&lt; 5</td>
<td>–</td>
</tr>
<tr>
<td>Range</td>
<td>5–61.8</td>
<td>5.3–34.8</td>
<td>–</td>
<td>–</td>
</tr>
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</table>

*EASI — Eczema Area and Severity Index

Figure 1. Percentage of lymphocytes CD4+CD25+ in peripheral blood in patients with allergic contact dermatitis and control group

Figure 2. Percentage of CD4+ lymphocytes with high expression of CD25 in patients with allergic contact dermatitis and control group

**Correlative correlation between the percentage of CD4+CD25+ cells and CD4+CD25high (p < 0.001)** (Figure 3). In patients with disseminated acute ACD, the percentage of both CD4+CD25+ and CD4+CD25high cells in peripheral blood varied depending on the duration time of skin lesions. In patients in whom the skin lesions lasted one week or less, the blood populations of CD4+CD25+ and CD4+CD25high cells were lowest. In patients examined in subsequent weeks of the disease, the population of CD4+CD25+ cells was higher during three weeks of the disease, whereas the elevated percentage of CD4+CD25high cells was seen in patients examined in the third week of the disease (Figures 4 and 5). The values of both CD4+CD25+ and CD4+CD25high populations were significantly higher in patients with widespread lesions (EASI > 16 scores) as compared with persons with a single skin lesion, (p < 0.05 and p < 0.01, respectively) (Figures 6 and 7). Patients with acute disseminated ACD showed significantly decreased serum level of TGF-β1 as compared with healthy persons and the remaining
eczema patients, whereas IL-10 mean serum level did not differ in all groups examined (Tables 2 and 3).

Discussion

Using the Fluorescence Activated Cell Sorting (FACS) method, it has been stated that the values of the CD4+CD25<sup>high</sup> cells range from 1.7% [14] to 2.1% [26] of CD4+CD25<sup>+</sup> T lymphocytes in the peripheral blood of an adult human. Similar values have been found in examined healthy controls, as well as in ACD patients with a localized form of disease, and in patients in remission.

Recent studies have shown that in healthy persons the lack of contact allergy results in active suppression of hapten-specific effector cells through regulatory CD4<sup>+</sup> cells secreting IL-10. Lymphocytes CD4<sup>+</sup> specific to nickel were ascertained both in people with an allergy to nickel and in healthy people, but specific CD8<sup>+</sup> lymphocytes have been found only in nickel allergy patients [20, 27]. In further experiments, Cavani et al. showed that CD4<sup>+</sup> cells isolated from healthy people did not proliferate after nickel stimulation in vitro, whereas removing the subpopulation of CD4<sup>+</sup>CD25<sup>+</sup> retrieved this ability [28].
Table 2. Mean serum level of IL-10 in patients with allergic contact dermatitis and control group

<table>
<thead>
<tr>
<th>Concentration of IL-10 [pg/ml]</th>
<th>Patients with disseminated ACD</th>
<th>Patients with limited ACD (al)</th>
<th>Control group (c)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute (ad)</td>
<td>Remission (rd)</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>4.84 ± 3.2</td>
<td>3.6 ± 2.8</td>
<td>3.5 ± 2.6</td>
<td>4.21 ± 2.9</td>
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</table>

Table 3. Mean serum level of TGF-β1 in patients with allergic contact dermatitis and control group

<table>
<thead>
<tr>
<th>Concentration of TGF-β1 (pg/ml)</th>
<th>Patients with disseminated ACD</th>
<th>Patients with limited ACD (al)</th>
<th>Control group (c)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute (ad)</td>
<td>Remission (rd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>22,821 ± 17,732</td>
<td>29,234 ± 22,906</td>
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</table>

In the ACD patients with acute stage disease, the percentage of peripheral blood CD4+CD25high cells was significantly higher than in healthy controls and in the remaining groups of contact eczema patients. To the best of our knowledge, no similar studies of ACD patients have been performed. In the scarce literature data regarding patients with atopic dermatitis (AD), an increased number of Tregs has been found in peripheral blood [29, 30], while results of another study showed a diminished population of Tregs [31].

Subpopulation of CD4+CD25+ can represent both regulatory T cells and activated effector CD4+ T cells, which may also transiently express the CD25 molecule [11, 32], so that only CD4+CD25high population should be taken into account as Tregs.

It is difficult to determine the source of Tregs detected in the blood of patients with ACD, but it is possible that CD4+CD25high appear on the periphery from CD4+CD25+ or from CD4+CD25+ cells after antigen stimulation, which shows the earlier growth of the total population of CD4+ (data not shown) and CD4+CD25+ than CD4+CD25high cells. The percentages of both CD4+ and CD4+CD25+ populations were significantly higher in the peripheral blood of ad ACD patients examined in the second and third weeks of the disease compared to the earlier values, whereas a significantly increased percentage of CD4+CD25high was seen later in the natural course of ACD. A study of skin specimens from the area of positive patch tests which were examined in consecutive days after allergen challenge [33], showed that skin infiltration of CD4+ cells preceded that of CD4+CD25+ and thus confirmed the above-mentioned observations. This may mean a delayed inflow of CD4+CD25+ cells from the blood, or it may suggest that they can emerge in the skin during 48 hours from these CD4+ lymphocytes T, which penetrated skin earlier. However, it has been recently shown that Tregs reside in normal human skin, from where they can be isolated and expanded. These cells express high levels of CD25, L-selectin, GITR, FoxP3, CTLA-4 and CLA [34]. Another study using immunohistochemical staining of skin specimens from human ACD lesions found that a significant number of epidermal as well as dermal CD3+, CD4+ and CD25+ cells were FoxP3+ [32]. In the most recent study, Tomura et al. showed in the murine CHS model a marked increase of CD4+ Tregs after allergen challenge both in the skin and in draining lymph nodes. The authors found that, in steady state, Tregs recirculate bidirectionally between skin and lymph nodes and that this Tregs traffic is substantially enhanced during CHS reaction, especially in the elicitation phase. These results may indirectly explain the increased amount of CD4+CD25high in the peripheral blood of our examined patients with acute disseminated ACD. These detected cells may be of the Tregs recirculating to skin from DLNs. Tomura et al. demonstrated that skin-derived Tregs have a stronger inhibitory effect on hapten-specific T-cell proliferation than LN-resident Tregs, and they inhibit local cutaneous immune response in situ. In a further study, the authors found that Tregs migrating from the skin (but not from LN) contain a fraction of CD4+CD25high population with strong immunosuppressive activity. These cells were seen in the challenged local skin but few in the unchallenged skin [35].

The role of CD4+CD25high in the course of ACD inflammatory reaction is supported also by the fact of dependence between the percentage of

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CD4⁺CD25high and degree of the skin lesions extension. In patients with considerable skin lesions (EASI ≥ 16 scores), the percentage of peripheral blood CD4⁺CD25high was significantly higher than in those with a mild form of the disease (p < 0.01).

The development of skin lesions, despite the higher percentage of CD4⁺CD25high, may indicate an insufficient role and the breakdown of the mechanisms related to Tregs, which suppress the inflammatory process in the skin. The increased population of Tregs CD4⁺CD25high is not translated simply into their activity, which suggests the presence of their functional defect or potent mechanisms of suppression of their activity. Cavani et al. showed that Tregs originating from the blood of eczema patients exerted a decreased activity in suppressing hapten-specific answers of both CD4⁺ and CD8⁺ cells [28]. It has been shown that Tregs can block APC depending, hapten-specific effector reaction of T lymphocytes and probably also play a role in completing the eczema inflammatory skin reaction in ACD patients [33].

The significant role of IL-10 in the suppressive activity of Tregs is not yet fully established, because of discrepancies in the literature data. It has been shown that Tregs exert their activity by mechanisms depending on direct contact of cells, independently of cytokines [14, 36–38]. Other data suggests that Tregs are able to produce great amounts of IL-10, when antigenic stimulation proceeds in defined conditions [39–42]. In our patients, there were no significant differences in serum levels of IL-10 between ACD patients and healthy controls, nor between the remaining groups examined. There was also no correlation between serum concentrations of IL-10 and the percentage of CD4⁺CD25high, which could suggest either that in those patients the suppressive activity of Tregs is independent of IL-10 secretion, or that increased amount of this cytokine does not depend simply on the magnitude of the Tregs population. IL-10 can also originate from cells other than Tregs, and its activity can occur only locally.

There is also a lack of unequivocal data concerning the role of TGF-β in the suppressive activity of regulatory T lymphocytes. There is evidence for participation of that cytokine in maintenance of Tregs on the periphery and stimulation of their generation from native T lymphocytes. The surface expression of latency associated protein (LAP), the precursor of TGFβ, was shown on activated Tregs (stimulated by anti-CD3/CD28 antibodies). [43]. The marked role of soluble TGF-β, together with membrane CTLA-4, in providing the suppressor signal has been demonstrated [44]. In experiments on mice and in humans, the role of connected activity of CTLA-4 and membrane form of TGF-β (mTGF-β) in the blocking of proliferation and function of effector CD4⁺ and CD8⁺ T lymphocytes [45] has been shown. Experiments from recent years seem to confirm the role of TGF-β in the suppressive activity of CD4⁺CD25high T cells, both in humans and in animals [37, 46]. It has been shown that Tregs not only express the membrane inactive form of TGF-β, but also the active form of this cytokine [47].

ACD patients with the acute stage of disseminated form of the disease showed a significantly decreased mean serum level of TGF-β in comparison with healthy controls. The diminished level of serum TGF-β, despite the elevated percentage of CD4⁺CD25high lymphocytes T, may indicate an inability of Tregs to produce sufficient amount of TGF-β in ACD patients. It may also suggest that this cytokine can be produced by cells other than Tregs.

Data from other authors, and our results, indicate that the increased percentage of CD4⁺ lymphocytes T with expression CD25 molecule and with high expression of CD25, seen in patients with acute stage allergic contact dermatitis, as well as the dependence of a percentage of these cells on the extension of skin lesions, suggest a role for Tregs in regulating the course of ACD. The growing percentage of the CD4⁺CD25high during the course of allergic contact dermatitis may indicate their generation on the periphery during the effector phase of the disease. Development of the skin eczematous inflammatory lesions despite the increased population of T regulatory cells (CD4⁺CD25high) suggests functional defect of these cells or strongly expressed mechanisms blocking of their activity. It is possible that TGF-β participates in the suppressive activity of CD4⁺CD25high regulatory T lymphocytes in allergic contact dermatitis.

References


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