Frequency and phenotypic analysis of CD4+CD25+ regulatory
T cells in children with juvenile idiopathic arthritis

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Background and Purpose: The aim of this study was to investigate the frequency of CD4+CD25+ regulatory T cells and their phenotypic expression in peripheral blood of children with active and non-active juvenile idiopathic arthritis (JIA) and healthy controls, to determine if their frequency or phenotypic expression is involved in the immunoregulation of this disease.

Methods: From October 2004 to October 2005, 55 JIA patients and 55 age- and gender-matched healthy controls were enrolled in the study at National Taiwan University Hospital. Flow cytometry was used to determine the frequency of CD4+CD25+ and CD4+CD25hi in CD4+ T cells and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) expression on CD4+CD25+ by tricolor staining. Basic profiles, medication history, clinical symptoms and laboratory data were obtained by chart review and outpatient department interviews.

Results: There was no significant difference in expression of CD4+CD25+ T cells between patients with inactive JIA and normal controls (13.74 ± 3.25% vs 12.85 ± 3.68%, p>0.05). The expression of CD4+CD25hi T cells was significantly lower in inactive JIA patients than in normal controls (1.89 ± 1.01% vs 2.76 ± 1.28%, p<0.01). The expression of CTLA-4 on CD4+CD25+ T cells was also significantly lower in inactive JIA patients compared with controls (4.37 ± 2.02% vs 6.33 ± 2.57%, p<0.001).

Conclusion: We speculate that a decreased frequency of CD4+CD25hi regulatory T cells and lower level of CTLA-4 expression on CD4+CD25+ regulatory T cells might play a role in the immunoregulation of JIA.

Key words: Arthritis, juvenile rheumatoid; CD4-positive T-lymphocytes; Cytotoxic T lymphocyte antigen 4; Immunophenotyping; T-lymphocyte subsets

Introduction

The establishment of a full repertoire of pathogen-specific lymphocytes to protect the host and the function of the lymphocytes to recognize self-antigens sometimes concur. Some of these potentially autoreactive T cells capable of recognizing tissue-specific antigens escape negative selection in the thymus and are released into the periphery. They can be cloned from lymphocytes in the lymph nodes of mice and human circulation, they are exquisitely regulated, and their activation can result in autoimmune diseases [1].

To maintain peripheral tolerance, the immune system has developed several mechanisms to suppress or regulate immunity in order to protect the body from sustained harmful immune responses. The failure of this regulatory network can result in autoimmune disease [2,3].

CD4+CD25+ regulatory T (Treg) cells play a key role in the regulation of immune responses [4]. Treg cells, which are also part of the normal human immune repertoire, control auto-aggressive T and B cells in humans [5,6]. They can block both the activation and the effector function of autoreactive T cells that escape other mechanisms of tolerance in human peripheral blood [5-7]. Some recent studies have identified a subset of CD4+CD25+ Treg cells that exhibit strong in vitro regulatory ability in a similar way to murine CD4+CD25+...
Treg cells [8,9]. This CD4+CD25hi T cell subset in humans comprises about 2% of circulating CD4+ T cells and inhibits the proliferation and secretion of cytokines [10]. It is not known, however, whether the frequency of this subset is lower in humans with autoimmune diseases.

Co-stimulatory or accessory molecules on CD4+CD25+ Treg cells may be related to Treg cell-induced suppression of immunity. These molecules are potent modulators of Treg cell development, homeostasis, and suppressor function [11]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), is constitutively expressed on naïve Treg cells and is suspected of playing a critical role in Treg cell function [12]. CTLA-4-deficient mice are known to develop fatal lymphoproliferative disease and immune dysregulation of multiple organs [13], and CTLA-4 has been shown to block the ability of Treg cells to suppress autoimmunity in an in vivo model of colitis [14].

Juvenile idiopathic arthritis (JIA), one of the most common rheumatic diseases in children, is defined as swelling and tenderness occurring in one or more joints in children under the age of 16 years with arthritis, and lasting longer than 6 weeks [15]. It is a heterogeneous group of conditions of unknown etiology, each of which has specific clinical features and prognostic implications [16]. The course and outcome of JIA can vary greatly, ranging from full recovery to lifelong symptoms and significant disability [17,18]. The chronic nature of the inflammatory process in JIA suggests a disturbance in immune regulation, which may be a result of an excessive inflammatory response co-occurring with a deficiency in control mechanisms.

This study compared the frequency of CD4+CD25+ and CD4+CD25hi Treg cells and CTLA-4 expression on Treg cells in peripheral blood of patients with active and inactive JIA and healthy controls. We also investigated whether differences in frequencies of these markers correlate with the different clinical courses by subdividing the study group into oligoarticular JIA, polyarticular JIA and systemic JIA.

Methods

Patients and control subjects
We enrolled 55 newly-diagnosed JIA patients treated at the National Taiwan University Hospital between 1990 and 2004 into this study. Each patient was diagnosed as having JIA in our hospital, received regular oral medication, and was followed up in our outpatient department for at least 1 year. The diagnosis and classification of JIA were based on the revised classification criteria for JIA [19], which categorizes oligoarticular JIA into two types: persistent oligoarticular type (per-OA JIA) and extended oligoarticular type (ext-OA JIA). We also enrolled 55 healthy age- and gender-matched controls.

We excluded patients and healthy controls who had acute asthma or other acute allergic diseases, including allergic rhinitis, atopic dermatitis, and urticaria, as well as other inflammatory or infectious conditions in the 2 weeks leading up to study enrollment to prevent the effect of other inflammatory diseases. We also excluded patients receiving etanercept therapy [20].

To analyze the dynamic changes of the above markers, we classified the JIA patients into inactive and active groups. The inactive group had forty three patients, and the active group twenty four. Twelve of the patients were categorized as sometimes inactive or sometimes active, depending on stage of disease at each sampling.

Active JIA (acute exacerbation stage) was defined based on having a clinical history of active arthritis symptoms or diagnosis by pediatric rheumatologists based on joint swelling or pain. In addition to joint swelling, the patients should have at least one of the other symptoms, including tenderness, erythema, heat, and limited range of motion of the involved joints. Inactive JIA (chronic stable stage) was defined by the absence of active arthritis symptoms and signs, although there might be mild elevation in erythrocyte sedimentation rate and complement level (C3, C4) due to the chronicity of inflammation.

Medical records were retrospectively reviewed from the follow-up period until the latest date of blood sampling. The JIA subtype, clinical course, and medications used in the last month were all recorded. Age at onset was defined as the age of the first physical symptoms or sign consistent with the diagnosis of JIA. Disease duration was defined as the time period between the age at onset and the age at blood sampling or clinical remission. Clinical remission was defined as the absence of active arthritis symptoms and signs, for at least 6 consecutive months without any medication, including non-steroidal anti-inflammatory drugs, steroids, and disease-modifying antirheumatic drugs. The demographic characteristics of the subjects, their disease status, and current medication are summarized in Table 1.
Monoclonal antibodies

Cell staining was performed using mouse anti-human monoclonal antibodies (mAbs) fluorescein isothiocyanate (FITC), phycoerythrin (PE) and peridinin chlorophyll protein (Per-CP) conjugate. The following mAbs were used: CD25 (immunoglobulin G [IgG]1, FITC, clone 2A3); CD4 (IgG1, Per-CP, clone SK3); and CTLA-4 (IgG2a kappa, PE, clone BNI3). The peripheral blood was stained with FITC-conjugated anti-CD25 (FL1), PE-conjugated anti-CTLA-4 (FL2) and Per-CP-conjugated anti-CD4 (FL3). To avoid non-specific Fc receptor staining, we used appropriate isotype controls of mouse anti-human mAbs: IgG1 (FITC, clone X40) for CD25 and IgG2a kappa (PE, clone G155-178) for CTLA-4 (CD152). All mAbs were purchased from Becton Dickinson Immunocytometry System (BD Biosciences, San Jose, CA, USA).

Sample preparation and flow cytometry analysis

Fresh blood was obtained by venipuncture and flow cytometry experiment was performed within 8 h of sampling to maintain viable cells and ensure accurate flow cytometry data. Three-color fluorochrome-conjugated monoclonal antibody sets were used to stain T cell surface markers. All antibodies were used at concentrations titrated for optimal staining according to manufacturer’s instructions. Briefly, an aliquot of whole blood was stained with the conjugated mAb or appropriate isotype control mAb and incubated for 30 min in the dark at 4°C. Erythrocytes were lysed with FACSM™ lysing solution (BD Biosciences), mixed thoroughly, and incubated for 15 min in the dark at 4°C. Samples were centrifuged at 2000 rpm for 10 min.

The supernatant was removed and washed twice with fluorescence-activated cell sorter (FACS) washing buffer (1% phosphate-buffered saline, 2% fetal calf serum, 0.05% sodium azide, 0.5M ethylenediamine tetra-acetic acid) and the cells were re-suspended with FACS washing buffer. Flow cytometry was performed with the FACS system (BD Biosciences) and all of the data were subsequently analyzed and displayed using Cell QuestPro software (BD Biosciences). Flow cytometric analysis between JIA patients and age-matched controls was performed in case-control paired fashion.

Polymorphonuclear leukocytes (neutrophils), lymphocytes, and monocytes exhibited characteristic light scatter properties that could be identified on a forward versus side light scatter plot with the use of Cell QuestPro data acquisition and analysis software. Lymphocyte populations were located using these parameters, and a live analysis gate (Gate 1 = Region 1, G1 = R1) was set around this population (Fig. 1A and 1B). Large, activated T cells were excluded.

Table 1. Demographic and clinical characteristics and current medication use of patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 55)</th>
<th>Inactive JIA (n = 43)</th>
<th>Active JIA (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M) [ratio]</td>
<td>20/35 (0.57)</td>
<td>16/27 (0.59)</td>
<td>9/15 (0.6)</td>
</tr>
<tr>
<td>Age (years; mean ± SD) [range]</td>
<td>12.35 ± 5.24 (5-25)</td>
<td>13.67 ± 5.50 (5-25)</td>
<td>12.06 ± 5.67 (5-25)</td>
</tr>
<tr>
<td>Age at onset (years; mean ± SD) [range]</td>
<td>NA</td>
<td>8.86 ± 3.95 (2-15)</td>
<td>8.05 ± 2.98 (2-14)</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent oligoarticular JIA</td>
<td>NA</td>
<td>16 (37.2)</td>
<td>9 (37.5)</td>
</tr>
<tr>
<td>Extended oligoarticular JIA</td>
<td>NA</td>
<td>10 (23.3)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Polyarticular JIA</td>
<td>NA</td>
<td>11 (25.6)</td>
<td>6 (25.0)</td>
</tr>
<tr>
<td>Systemic JIA</td>
<td>NA</td>
<td>6 (14.0)</td>
<td>6 (25.0)</td>
</tr>
<tr>
<td>Human leukocyte antigen B27</td>
<td>NA</td>
<td>13 (30.2)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>NA</td>
<td>4 (9.3)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>CRP (μg/L; mean ± SD) [range]</td>
<td>NA</td>
<td>26 ± 23 (0-78)</td>
<td>313 ± 298* (81-1047)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>NA</td>
<td>12 (27.9)</td>
<td>9 (37.5)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>NA</td>
<td>22 (51.2)</td>
<td>11 (45.8)</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>NA</td>
<td>19 (44.2)</td>
<td>15 (62.5)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>NA</td>
<td>3 (7.0)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>NA</td>
<td>3 (7.0)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>NSAID</td>
<td>NA</td>
<td>37 (86.0)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Disease duration (years; mean ± SD) [range]</td>
<td>NA</td>
<td>4.54 ± 3.32 (1-14)</td>
<td>3.95 ± 4.26 (1-14)</td>
</tr>
</tbody>
</table>

Abbreviations: JIA = juvenile idiopathic arthritis; F = female; M = male; SD = standard deviation; CRP = C-reactive protein; NSAID = non-steroidal anti-inflammatory drugs; NA = not applicable

*p<0.05 between active and inactive disease.
Fig. 1. Comparison of flow cytometric analysis of CD4+CD25+ or CD4+CD25hi cell populations and of surface expression of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) on CD4+CD25+ T cells between controls (A) and inactive juvenile idiopathic arthritis patients (B). Analysis gates show the percentages of CD25+ (C) and CD25hi (D) in CD4+ T cells and percentages of CTLA-4 expression on CD4+CD25+ T cells (E, F). Isotype controls for each sample were run in conjunction with CD25 staining and with CTLA-4 (immunoglobulin G2a) staining, respectively (data not shown). G1 = gate 1; R1 = region 1; UR = upper limit; R2 = region 2; R3 = region 3.
The gating strategy is used to analyze surface CD25+CD4− regulatory T cells in JIA patients, 12 of whom changed status over time, and 55 healthy controls. Their demographic characteristics, basic profiles, disease status, and current medications were summarized in Tables 1 and 2. Within the study group, patients with active JIA were not significantly different from those with inactive JIA with regard to gender ratio, age, onset age, subtype, human leukocyte antigen B27, rheumatoid factor, percentage of current medication use, and disease duration (Table 1).

**Results**

**Demographic and clinical characteristics of subjects**

This study included 43 inactive and 24 active JIA patients, 12 of whom changed status over time, and 55 healthy controls. Their demographic characteristics, basic profiles, disease status, and current medications were summarized in Tables 1 and 2. Within the study group, patients with active JIA were not significantly different from those with inactive JIA with regard to gender ratio, age, onset age, subtype, human leukocyte antigen B27, rheumatoid factor, percentage of current medication use, and disease duration (Table 1).

**Frequency of CD4+CD25+ cells in CD4+ T cells**

Active JIA patients had a significantly higher percentage of CD4+CD25+ cells than the healthy controls (15.48 ± 3.74 vs 12.85 ± 3.68, p<0.01). The difference in this measure was not significant between either the inactive JIA patients and healthy controls (13.74 ± 3.25 vs 12.85 ± 3.68, p>0.05) or the active JIA and inactive JIA patients (15.48 ± 3.74 vs 13.74 ± 3.25, p>0.05) [Fig. 2A].

**Frequency of CD4+CD25hi cells in CD4+ T cells**

Inactive JIA patients had a significantly lower percentage of CD4+CD25hi cells than the healthy controls (1.89 ± 1.01% vs 2.76 ± 1.28%, p<0.01), and active JIA patients had a significantly higher percentage of CD4+CD25hi cells than the inactive JIA patients (2.89 ± 1.56 vs 1.89 ± 1.01, p<0.05) [Fig. 2B].

**Table 2.** Age, gender, age at onset, human leukocyte antigen B27, rheumatoid factor and current medication use in subtypes of inactive juvenile idiopathic arthritis (JIA) patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Persistent oligoarticular JIA (n = 16)</th>
<th>Extended oligoarticular JIA (n = 10)</th>
<th>Polyarticular JIA (n = 11)</th>
<th>Systemic JIA (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M [ratio])</td>
<td>5/11 (0.45)</td>
<td>4/6 (0.67)</td>
<td>3/8 (0.38)</td>
<td>4/2 (0.67)</td>
</tr>
<tr>
<td>Age (years; mean ± SD) [range]</td>
<td>12.54 ± 5.58 (5-25)</td>
<td>15.44 ± 4.98 (9-25)</td>
<td>12.86 ± 4.45 (5-19)</td>
<td>15.27 ± 7.83 (7-24)</td>
</tr>
<tr>
<td>Age at onset (years; mean ± SD) [range]</td>
<td>8.15 ± 4.04 (2-14)</td>
<td>9.67 ± 3.20 (5-15)</td>
<td>9.26 ± 3.76 (3-14)</td>
<td>8.68 ± 5.69 (2-15)</td>
</tr>
<tr>
<td>Human leukocyte antigen B27</td>
<td>8 (50.0)</td>
<td>5 (50.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>2 (12.5)</td>
<td>0 (0.0)</td>
<td>2 (18.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>4 (25.0)</td>
<td>3 (30.0)</td>
<td>3 (27.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>9 (56.3)</td>
<td>6 (60.0)</td>
<td>4 (36.4)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>9 (56.3)</td>
<td>5 (50.0)</td>
<td>5 (45.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0 (0.0)</td>
<td>1 (10.0)</td>
<td>2 (18.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td>NSAID</td>
<td>12 (75.0)</td>
<td>10 (100.0)</td>
<td>10 (90.9)</td>
<td>5 (83.3)</td>
</tr>
</tbody>
</table>

Disease duration (years; mean ± SD) [range]

Abbreviations: F = female; M = male; SD = standard deviation; NSAID = non-steroidal anti-inflammatory drugs
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CTLA-4 phenotypic expression on CD4+CD25+ T cells
The inactive JIA patients had a significantly lower expression of CTLA-4 on Treg cells than the healthy controls (4.37 ± 2.02% vs 6.33 ± 2.57%, p<0.001). However, there was no significant difference in this measure between active and inactive JIA patients (5.31 ± 1.81% vs 4.37 ± 2.02%, p>0.05) [Fig. 2C].

Fig. 2. Comparison of the frequency of (A) CD4+CD25+ and (B) CD4+CD25hi regulatory T cells and (C) cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) expression on CD4+CD25+ regulatory T cells between control subjects, inactive juvenile idiopathic arthritis (JIA) patients and active JIA patients. Dots scatter plot; the horizontal line within the vertical points marks the median [1.7% for patients with inactive JIA vs 2.73% for patients with active JIA and 2.64% for control subjects in (B), respectively]. NS = not significant; *p<0.05.

Frequency of CD4+CD25hi/CD4+CD25hi cells in CD4+ T cells and CTLA-4 expression on CD4+CD25hi T cells
When using the Kruskal-Wallis test to analyze intragroup differences, we found a significant difference only in percentage of CD4+CD25hi cells (overall p=0.00004) [Fig. 3]. The patients with per-OA JIA had a significantly higher percentage of CD4+CD25hi cells than either those with poly-JIA (2.70 ± 0.70% vs 1.41 ± 1.00%, p<0.01) or those with systemic JIA (2.70 ± 0.70% vs 1.03 ± 0.46%, p<0.01) [Fig. 3B].

Discussion
In this study, we compared the frequency of CD4+CD25hi and CD4+CD25hi Treg cells and CTLA-4 expression on Treg cells in the peripheral blood of patients with active and inactive JIA and healthy controls. We found no significant difference in frequency of CD4+CD25hi T cells
between patients with inactive JIA and normal controls, a significantly lower frequency of CD4+CD25hi T cells in inactive JIA patients compared to normal controls, and a significantly lower frequency of CTLA-4 expression on CD4+CD25+ T cells in inactive JIA patients compared to controls. Our intragroup study found the only significant difference to be in frequency of CD4+CD25hi cells, with patients with per-OA JIA having a significantly higher percentage of these cells than those with polyarticular JIA or systemic JIA.

Kojima and Prehn discovered that due to the loss of a CD4+CD25+ Treg cell population in peripheral lymphoid tissues, thymectomy in 3-day-old mice led to the development of multiorgan autoimmune disease [21], Sakaguchi and colleagues showed that 5-10% of CD4+ T cells, which co-expressed the interleukin-2 receptor alpha chain (CD25), were needed to control autoreactive T cells in vitro [22-24]. Animal studies have found that CD4+CD25+ Treg cells are able to inhibit autoimmune diabetes [25], prevent inflammatory bowel disease [14], prevent the expansion of other T cells in vivo [26], and inhibit T cell activation in vitro [27]. Although Treg cells are thought to play an important role in the pathogenesis of autoimmune diseases...
in animal models, their role in human autoimmune disease has not yet been thoroughly demonstrated. Although a lymphocyte population with identical phenotypic and functional properties has recently been identified in the human thymus and peripheral blood [9,10], many questions about whether and how Treg cells can actively regulate autoimmune diseases in humans still need to be answered.

The human regulatory or suppressive functions of Treg cells have been observed to occur only when the cells express high levels of CD25, which are distinct from the CD25+ T cells [10]. Recently, FoxP3 has been identified as a specific marker of Treg cells, distinguishing them from recently activated, non-Treg cells [28,29]. One study of JIA reported the mean expression of FoxP3 mRNA to be significantly higher in CD4+CD25bright than in CD4+CD25+ T cells, and its expression seemed to correlate well with the clinical subtypes and clinical courses of JIA [30]. Hence, the main subsets involved in the regulatory function of Treg cells are in the CD25hi T cells. Our data on the frequency of CD4+CD25+, CD4+CD25hi cells in peripheral blood, healthy controls were consistent with the above study, causing us to wonder whether or not this reduced frequency of CD4+CD25hi cells contributes to the development of JIA.

In some studies of the frequency of CD4+CD25+, CD4+CD25hi cells in peripheral blood, adult systemic lupus erythematosus patients had significantly lower levels of Treg cells than normal controls [31], and type 1 diabetes mellitus patients had significantly lower levels of Treg cells than normal controls [32], although one study of peripheral blood in adults with rheumatoid arthritis showed significantly higher levels of Treg cells in CD4+ T cells than in normal controls [33]. Many previous studies detecting and isolating Treg cells in humans have been complicated by the fact that CD25 is a marker of recent T cell activation. However, none of the above studies classified patients into acute exacerbation (active) or chronic stable (inactive) stages and compared the difference. Our study classified JIA patients into inactive and active groups to avoid acute inflammation-induced increases in CD25 expression when comparing inactive JIA patients and healthy controls. Therefore, our finding that the frequency of CD4+CD25+ T cells was significantly higher in patients with active JIA than healthy controls may only indicate an activation-induced increase in CD25 expression.

The presence and enrichment of CD4+CD25bright T cells has been found in the synovial fluid of human joints with rheumatoid arthritis when compared to their peripheral blood, and that these T cells were able to suppress reactive T cells, leading the investigators to speculate that the enriched functional CD4+CD25bright Treg cells in inflamed joints exerted an immunosuppressive effect locally, and influenced disease activity and progression [33]. In a similar study of patients with JIA, CD4+CD25+ and CD4+CD25hi Treg cells expressed more FoxP3 mRNA in synovial fluid than peripheral blood, and they were found to regulate reactive T cells more actively in vitro coculture experiments [30]. Since the joint is the main target organ in JIA, Treg cell populations in synovial fluid can represent an active inflammation, suggesting the existence of a negative feedback system in which Treg cells are generated and triggered in the ongoing inflammation. Our data revealed that the greater frequency of CD4+CD25hi cells we found in active JIA compared to inactive JIA patients may be the result of a similar compensatory mechanism.

JIA is not a homogenous disease, but consists of various subtypes with striking differences in both severity and outcome [19]. A much less favorable prognosis in JIA, often involving non-remitting destructive and disabling arthritis, requires aggressive immunotherapy. Therefore, we compared the frequencies of these measures in patients with per-OA JIA, a subtype with a mild self-limiting, remitting course of disease, with patients with poly-JIA and systemic JIA, subtypes with less favorable, non-self-remitting courses involving joint erosion or systemic involvement. The only significant difference this intragroup comparison found was in the frequency of CD4+CD25hi in CD4+ T cells. Patients with per-OA JIA had a higher frequency of CD4+CD25hi T cells than other subtypes, a finding that was similar to that of another study [30]. Therefore, the CD25hi subsets in Treg cells might play a major role in immunoregulation.

In mouse models, CTLA-4 blockade can reverse and abolish the suppressive function of Treg cells [12,14] and block CTLA-4 in vivo. This anti-CTLA-4 treatment can result in the abrogation of any protection offered by co-transfer of the CD4+CD25+ population in mice models of the autoimmunity of diabetes and intestinal inflammation [14,25]. Recently, it has been shown that the expression level of CTLA-4 in human CD4+CD25+ Treg cells correlates with their suppressive capacity [34,35]. Another recent
report has also proposed an indirect mechanism by which CD28 and CTLA-4 expression are involved in the function of Treg cells [36]. Although CTLA-4 expresses itself and functions on the cell surface, some studies directly analyzing human blood did not find CD4+CD25+ T cells to express CTLA-4 there [10]. A similar finding could be discovered in mice, in which CTLA-4 is constitutively expressed, mainly in cytoplasm of murine CD4+CD25+ Treg cells, but is rarely detected on the cell surface [14]. However, due to the longer kinetics of suppression, the peak levels of CTLA-4 expression can be induced on T cells by 2-3 days post-activation [37]. Our patients had chronic inflammation for at least 1 year, making it very likely that their T cells would be found with CTLA-4 and its subpopulations, including Treg cells. Because the kinetics of cell surface CTLA-4 parallel intracellular CTLA-4 [38], CTLA-4 expression on the surface of T cells can also be used as a measure of intracellular CTLA-4. The expression levels of CTLA-4 are lower on the Treg cells of inactive JIA patients than on the Treg cells of healthy controls, and the difference in CTLA-4 expression may play a role in the immunodysregulation of JIA.

In conclusion, a decreased frequency of CD4+CD25hi in CD4+ cells and a decreased expression of CTLA-4 on Treg cells may be related to the immunodysregulation of JIA. In different stages of JIA, we speculate that changes in phenotypes such as CD25+ on Treg cells, which lead to enhanced capacity to suppress autoimmunity, seem to be the mechanisms by which Treg cells regulate local or systemic inflammation. These suggested explanations should be interpreted with great caution, however, because the number of patients with active JIA in our study was limited and because the expression of CD25 may also be increased after T cell activation. The frequency of CD4+CD25hi T cells may regulate the clinical course. CD4+CD25hi seems to play more of a role in the limiting and even reversal of established autoimmune pathology of per-OA JIA than in other subtypes of JIA. However, despite the highly significant difference in frequency of CD4+CD25hi between per-OA and other subtypes of JIA, its frequency cannot be used to predict whether oligoarticular JIA will progress to a less favorable outcome or persist as a per-OA stage. A definite conclusion in this regard is limited by our study’s small number of patients with systemic JIA and limited follow-up periods. We also did not perform functional assays of Treg cells in any of the above studies. The role of autoreactive T cells in the pathogenesis of JIA is unknown. While CD25+ and CTLA-4 expression on Treg cells may be associated with the immunoregulation of JIA, there are many possible mechanisms needing further investigation.

References


