Review Article

The Proportion of Regulatory T Cells in Patients with Systemic Lupus Erythematosus: A Meta-Analysis

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Received 26 March 2018; Revised 21 July 2018; Accepted 31 July 2018; Published 3 September 2018

Academic Editor: Keshav Raj Sigdel

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Background. Accumulating evidence indicates that a deficiency in or dysfunction of regulatory T cells (Tregs) is involved in the pathogenesis of systemic lupus erythematosus (SLE). As different markers have been used to identify Tregs, recent studies on the proportions of Tregs in SLE patients have generated controversial results. To clarify the status of Tregs in such patients, we determined the proportions of Tregs present during development of the disease, with special consideration of controversial cellular markers. Methods: We identified studies reporting the proportions of Tregs in SLE patients by searching relevant databases through March 2018. Using the PRISMA guidelines, we performed a random effects meta-analysis of the frequencies of Tregs defined in different ways. Inconsistency was evaluated using the I-squared index (I²), and publication bias was assessed by examining funnel plot asymmetry using the Begger and Egger tests. Results. Forty-four studies involving 2779 participants were included in the meta-analysis. No significant difference in the proportions of Tregs was evident between 1772 patients and 1007 controls [-0.191, (-0.552, 0.362), p = 0.613, I² = 95.7%]. We next conducted subanalyses based on individual definitions of Tregs. When the Treg definition included “FOXP3-positive” cells, the proportions did not differ between SLE patients and controls [-0.042, (-0.548, 0.632), p = 0.889, I² = 96.6%]; this was the case when Tregs were defined as either “CD25low/FOXP3+” or “CD25high/FOXP3+” cells. SLE patients had lower proportions of Tregs that were “single CD25-positive” [-1.428, (-1.982, -0.873), p < 0.001, I² = 93.4%] and “CD127-negative” [-1.093, (-2.002, -0.183), p = 0.018, I² = 92.6%] compared to controls. Tregs defined as “CD25bright”, “CD25bright/highCD127low/−”, and “CD25highCD127low/FOXP3+” did not differ in proportion between SLE patients and controls. Conclusions. The Treg proportions varied by the cellular identification method used. The proportions of Tregs that were accurately identified and functionally validated fell among patients with SLE. Stricter definitions of Tregs are necessary when evaluating the status of such patients.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by highly variable clinical manifestations associated with widespread inflammation and overproduction of autoantibodies [1]. Growing evidence suggests that regulatory T cells (Tregs) maintain peripheral tolerance by controlling and limiting harmful immune responses [2]. Failure to maintain appropriate numbers of functional Tregs plays an important role in SLE pathogenesis [3]. However, initial studies of Treg status in the peripheral blood (PB) of patients with SLE have generated controversial results. One reason for the inconsistencies is that multiple phenotypes of Tregs are identified using different markers [3]. Tregs were first described as a peripheral CD4+ subpopulation
expressing interleukin-2 receptor alpha chains (CD25) [4]. Further studies revealed that CD25 was expressed not only on Tregs but also on activated cells lacking regulatory functions, although the CD4+ T cell subset expressed the highest levels of CD25 (CD4+CD25hi) and exhibited in vitro immunosuppressive features [5]. Forkhead box protein P3 (FOXP3), a transcription factor expressed at high levels in authentic Tregs, plays a key role in Treg development and is thought to be one of the most specific Treg cell markers [6]. However, the marker cannot be used to sort live cells as the protein is intracellular. In addition, CD127, the alpha chain of the IL-7 receptor, was reported to be upregulated on human T cells after activation and downregulated on Tregs [7], being inversely correlated with the FOXP3 expression level. Thus, co-staining for CD127 and CD25 is thought to be one of the most specific Treg markers [6]. However, the marker cannot be used to sort live cells as the protein is intracellular. In addition, CD127, the alpha chain of the IL-7 receptor, was reported to be upregulated on human T cells after activation and downregulated on Tregs [7], being inversely correlated with the FOXP3 expression level. Thus, co-staining for CD127 and CD25 has been proposed to efficiently discriminate between Tregs and activated T cells [8]. The available data on the proportions and phenotypes of Tregs of SLE patients are contradictory; more studies are required to better understand the role played by Tregs during the disease course.

Here, we meta-analyze reports documenting the proportion of Treg cells among CD4+ T cells in the PB of patients with active and inactive SLE, as well as healthy controls, to better understand Treg malfunctions in patients with SLE.

2. Methods

2.1. Data Sources and Searches. This meta-analysis was performed as suggested by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement and was registered at the International Prospective Register of Systematic Reviews (no. CRD42017060258) [9]. We searched for relevant studies published between January 1, 1950, and March 1, 2018, using PubMed, Embase, the Cochrane database, the Web of Knowledge, Clinical Trials.gov, and FDA.gov, with no restrictions in terms of the primary outcome or publication language. We used the MeSH terms "lupus erythematosus, systemic" and "T-lymphocytes, regulatory" and their combination. All potentially eligible studies were considered except for reviews and murine experiments. Key articles listed in the references were retrieved manually.

2.2. Study Selection and Data Extraction. The inclusion criteria were (1) evaluation of the proportion of Tregs among CD4+ T cells of SLE patients using the 1997 revised American College of Rheumatology criteria, (2) available as a full text article, and (3) information on the number of patients and controls. Two investigators independently selected and identified relevant publications, and a third investigator resolved any disagreements. The evidence levels of the studies were assessed based on the 2011 guidelines of the Oxford Center for Evidence-Based Medicine [10]. Quality assessment was done with the Newcastle-Ottawa Quality Assessment Scale, which can be used to assess the quality of nonrandomized studies [11].

We recorded patient baseline characteristics and their country of origin, the year of publication, the number of patients and controls, the definition of Tregs used (including CD25+, CD25bright, CD25high, CD25low/−, FOXP3+, FOXP3+, CD25FOXP3+, CD25+CD127+, CD25bright/CD127lo/−, and CD25high/CD127lo/−,−FOXP3+), and the mean (or median) and standard deviation (SD) of the proportion of Tregs among CD4+ T cells. Data on the proportion of Tregs in patients with active and inactive SLE were also extracted.

2.3. Statistical Analysis. For continuous outcomes (the proportions of Tregs among CD4+ T cells of patients with active and inactive SLE and healthy controls), we calculated standardized mean differences (SMDs) and compared these values using a random effects model (REM) (the DerSimonian and Laird method) [12]. When Treg percentages were reported as medians with interquartile ranges (IQRs), we calculated means and SD (SD = IQR/1.35) [13]. The Cochrane chi-squared test was used to explore between-study heterogeneity. As heterogeneity was high (I^2 > 75%), we drew forest plots and performed subgroup analyses to explore the possible effects of study characteristics on outcomes. Publication bias was assessed by examining funnel plot asymmetry using the Beggar and Egger tests (p ≥ 0.05). Preplanned sensitivity analysis was performed by omitting each study individually and calculating the remaining pooled effect. All statistical analyses were conducted using Stata software (ver. 12.0).

3. Results

3.1. Study Characteristics. We identified 2264 studies, of which 44 (with data on 1772 patients and 1007 controls) were included in analysis (Figure 1). The details are shown in Table 1. The average age of the SLE patients ranged from 8.7–45.4 years; the proportion of females ranged from 56.7–100%, the disease duration from 1.5–26.6 years, the average erythrocyte sedimentation rate from 18.6–78.8 mm/hour, and the SLE Disease Activity Index (SLEDAI) from 2.0–17.4. Patients were treated with corticosteroids (CS) and immunosuppressants including cyclophosphamide (CTX), azathioprine (AZA), cyclosporin A (CsA), mycophenolate mofetil (MMF), and chloroquine (HCQ). All controls were healthy without any autoimmune disease. All studies were poor-quality case-control studies or case series; thus, they were all of evidence level 4. We regarded all studies as case-control studies and scored them using the Newcastle-Ottawa Quality Assessment Scale (NOQAS); all studies had a score of 3–5.

3.2. Proportion of Tregs in the PB of SLE Patients. We initially compared the proportion of Tregs in SLE patients and healthy controls regardless of the Treg definition used. Surprisingly, no significant difference was apparent in any study [−0.113, (−0.552, 0.362), p = 0.613]. Also, heterogeneity, as assessed by the I^2 statistic, was 95.7% (p < 0.001) and thus very high. The Egger test revealed no publication bias (t = 0.70, p = 0.491) (Figure 2).

We hypothesized that the primary reason for the unexpected results might be that the definitions of Tregs were inconsistent. Thus, we performed subgroup analysis based on the Treg definitions to explore the potential sources of...
Records identified through database searching \((n = 2259)\)

Additional records identified through manual search \((n = 5)\)

Records after duplicates were removed \((n = 773)\)

Records screened \((n = 773)\)

Full-text articles assessed for eligibility \((n = 379)\)

Studies included in qualitative synthesis \((n = 44)\)

Studies included in quantitative synthesis (meta-analysis) \((n = 44)\)

Records excluded on the basis of title and abstract \((n = 394)\)

Full-text articles excluded, with reasons \((n = 335)\)

(a) Not original articles (reviews) \((n = 78)\)

(b) Not human experiment \((n = 213)\)

(c) Not research about SLE patient \((n = 9)\)

(d) Conference abstract superseded by publication \((n = 1)\)

(e) Not report the proportion of Tregs among \(CD4^+\) T cells \((n = 32)\)

(f) No comparison group \((n = 2)\)

**Figure 1**: The study selection process.

heterogeneity. First, we analyzed studies that identified Tregs only as “CD25-positive” (Supplementary Figure 1). Pooled analysis of all 18 trials revealed a significant decrease in the proportion of Tregs in SLE patients compared to controls \([-1.428, \ (-1.982, \ -0.873), \ p < 0.001]\) with statistically significant between-study heterogeneity \(I^2 = 93.4\%, \ p < 0.001\) and publication bias detected by the Egger test \((t = -4.29, \ p = 0.001)\). In detail, we found significant differences in the proportion of Tregs between SLE patients and healthy controls when Tregs were defined as “CD25” cells \([-1.512, \ (-2.488, \ -0.535), \ p = 0.002]\) and as “CD25high” cells \([-1.074, \ (-1.830, \ -0.318), \ p = 0.005]\). However, in two studies, the proportion of Tregs defined as “CD25high” cells did not differ significantly between patients and healthy controls \([-3.495, \ (-9.197, \ 2.207), \ p = 0.230]\) (Table 2).

Second, we analyzed studies in which Tregs were defined as “FOXP3” cells (Supplementary Figure 2). Pooled analysis of all 36 trials revealed no significant difference in the proportion of such Tregs between SLE patients and controls \([0.042, \ (-0.548, \ 0.632), \ p = 0.889]\). Statistically significant heterogeneity was evident among the studies \(I^2 = 96.6\%, \ p < 0.001\). The Egger test detected no publication bias \((t = 0.81, \ p = 0.424)\). Among the studies, five used “CD25low/FOXP3” to define Tregs, and three simply “FOXP3”; the proportion of Tregs in SLE patients appeared to be higher than in controls \([5.409, \ (2.112, \ 8.705), \ p = 0.001; 1.101, \ (0.435, \ 1.768), \ p = 0.001, \ resp.]\). However, pooling of these data with those of other studies identifying Tregs as “CD25high/FOXP3” cells revealed a lower proportion of Tregs in patients than controls; Tregs were identified as “CD25/FOXP3” cells \([-1.279, \ (-2.079, \ -0.479), \ p = 0.00]\) and “CD25high/FOXP3” cells \([-0.663, \ (-1.289, \ -0.036), \ p = 0.038]\) (Table 2).

Finally, the other eight groups that used “CD127-negative” to define Tregs showed that such cell numbers decreased in SLE patients \([-1.093, \ (-2.002, \ -0.183), \ p = 0.018]\) with statistical heterogeneity \(I^2 = 92.6\%, \ p < 0.001\) and publication bias \((t = -3.05, \ p = 0.022)\). More specifically, pooling the data of four studies in which Tregs was identified as “CD25/CD127” cells revealed a significant difference between SLE patients and controls \([-1.093, \ (-2.002, \ -0.183), \ p = 0.018]\), but no significant difference was apparent
Table 1: Characteristics of the individual studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Author (ref.)</th>
<th>Publish year</th>
<th>Country</th>
<th>EL a</th>
<th>Q b</th>
<th>Case numbers</th>
<th>Tregs' definition</th>
<th>Data type</th>
<th>% of Tregs among CD4+ T cells [mean (or median) ± SD]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesquita, D., Jr., et al. [31]</td>
<td>2018</td>
<td>Brazil</td>
<td>4</td>
<td>4</td>
<td>37</td>
<td>CD4&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>6.49 ± 4.99 3.05 ± 1.76</td>
</tr>
<tr>
<td>Ferreira et al. [32]</td>
<td>2017</td>
<td>UK</td>
<td>4</td>
<td>5</td>
<td>34</td>
<td>CD25\textsuperscript{low}CD12\textsuperscript{low}&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>18.8 ± 2.64 6.39 ± 0.74</td>
</tr>
<tr>
<td>Singla et al. [33]</td>
<td>2017</td>
<td>USA</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>CD25&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>4.48 ± 0.49 2.97</td>
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<td>Wang et al. [34]</td>
<td>2017</td>
<td>China</td>
<td>4</td>
<td>5</td>
<td>47</td>
<td>CD25 &quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>2.32 ± 0.15 3.02 ± 0.34</td>
</tr>
<tr>
<td>Zahran et al. [35]</td>
<td>2016</td>
<td>Egypt</td>
<td>4</td>
<td>4</td>
<td>20</td>
<td>CD25\textsuperscript{high}&quot;FOXP3&quot;</td>
<td>Original</td>
<td>1.32 ± 0.25 2.24 ± 0.52</td>
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<tr>
<td>Margiotta et al. [36]</td>
<td>2016</td>
<td>Italy</td>
<td>4</td>
<td>3</td>
<td>13</td>
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<td>Original</td>
<td>1.27 ± 0.9 2.8 ± 1.2</td>
</tr>
<tr>
<td>Zabinska et al. [37]</td>
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<td>Poland</td>
<td>4</td>
<td>4</td>
<td>54</td>
<td>CD25&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>1.10 ± 1.27 3.36 ± 0.52</td>
</tr>
<tr>
<td>Handono et al. [38]</td>
<td>2016</td>
<td>Indonesia</td>
<td>4</td>
<td>5</td>
<td>62</td>
<td>CD25&quot;FOXP3&quot;</td>
<td>Original</td>
<td>2.3 ± 2.1 0.9 ± 0.8</td>
</tr>
<tr>
<td>Legorreta-Haquet et al. [39]</td>
<td>2016</td>
<td>Mexico</td>
<td>4</td>
<td>3</td>
<td>47</td>
<td>CD25\textsuperscript{low}CD12\textsuperscript{low}&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>1.54 ± 0.84 2.92 ± 0.73</td>
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<td>Egypt</td>
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<td>37</td>
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<td>Original</td>
<td>5.26 ± 4.2 45.6 ± 6.4</td>
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<td>Brazil</td>
<td>4</td>
<td>4</td>
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<td>0.74 ± 0.34 1.83 ± 0.77</td>
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<td>Greece</td>
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<td>Austria</td>
<td>4</td>
<td>4</td>
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<td>Original</td>
<td>5.1 ± 0.5 1.1 ± 0.2</td>
</tr>
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<td>2014</td>
<td>Poland</td>
<td>4</td>
<td>4</td>
<td>21</td>
<td>CD25\textsuperscript{high}&quot;FOXP3&quot;</td>
<td>Original</td>
<td>18.57 ± 10.44 32.08 ± 11.54</td>
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<tr>
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<td>United Kingdom</td>
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<td>3</td>
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<td>Original</td>
<td>6.75 ± 3.73 6.65 ± 1.59</td>
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<td>Prado et al. [46]</td>
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<td>Spain</td>
<td>4</td>
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<td>FOXP3&quot;</td>
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<td>13</td>
<td>CD25&quot;FOXP3&quot;</td>
<td>Calculated</td>
<td>6.71 ± 3.57 3.13 ± 1.61</td>
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<td>2012</td>
<td>China</td>
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<td>CD25\textsuperscript{low}&quot;FOXP3&quot;</td>
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<td>4.6 ± 1.3 5.0 ± 1.3</td>
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<td>1.53 ± 0.8 3.97 ± 1.21</td>
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<td>Calculated</td>
<td>0.97 ± 1.04 0.66 ± 0.50</td>
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<td>Data type</td>
<td>% of Tregs among CD4+ T cells [mean (or median) ± SD]</td>
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<td>Portugal</td>
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<td>4</td>
<td>34</td>
<td>CD25^{bright}CD127^{low/−}</td>
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<td>8.16 ± 3.53 7.10 ± 2.70</td>
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<td>China</td>
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<td>4</td>
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<td>0.64 ± 0.39 0.86 ± 0.39</td>
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<td>2009</td>
<td>Austria</td>
<td>4</td>
<td>4</td>
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<td>Original</td>
<td>7.5 ± 1.0 1.4 ± 0.4</td>
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<td>2009</td>
<td>Egypt</td>
<td>4</td>
<td>4</td>
<td>30</td>
<td>CD25^{negative}</td>
<td>Calculated</td>
<td>6.18 ± 1.90 8.07 ± 2.04</td>
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<td>Li et al. [57]</td>
<td>2009</td>
<td>China/Chinese</td>
<td>4</td>
<td>4</td>
<td>47</td>
<td>CD25^{high}FOXP3^{+}</td>
<td>Calculated</td>
<td>3.37 ± 1.83 3.5 ± 1.4</td>
</tr>
<tr>
<td>Lee et al. [58]</td>
<td>2008</td>
<td>Korea</td>
<td>4</td>
<td>3</td>
<td>20</td>
<td>CD25^{+}</td>
<td>Original</td>
<td>15.2 ± 0.2 22.1 ± 0.9</td>
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<td>Venigalla et al. [59]</td>
<td>2008</td>
<td>Germany</td>
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<td>4</td>
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<td>10.68 ± 1.63 6.3 ± 0.4</td>
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<td>58</td>
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<td>2.35 ± 0.51 1.75 ± 0.10</td>
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<td>2008</td>
<td>Austria</td>
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<td>3</td>
<td>17</td>
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<td>Calculated</td>
<td>1.15 ± 1.00 2.0 ± 0.1</td>
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<td>Azab et al. [62]</td>
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<td>Egypt</td>
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<td>4</td>
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<td>CD25^{−}</td>
<td>Calculated</td>
<td>1.06 ± 0.40 1.8 ± 0.16</td>
</tr>
<tr>
<td>Hu et al. [63]</td>
<td>2008</td>
<td>China</td>
<td>4</td>
<td>4</td>
<td>38</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>13.02 ± 3.60 6.5 ± 1.34</td>
</tr>
<tr>
<td>Yan et al. [64]</td>
<td>2008</td>
<td>China</td>
<td>4</td>
<td>3</td>
<td>25</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>10.37 ± 4.44 7.78 ± 4.69</td>
</tr>
<tr>
<td>Hahn et al. [66]</td>
<td>2008</td>
<td>America</td>
<td>4</td>
<td>4</td>
<td>36</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>8.00 ± 1.64 4.78 ± 0.43</td>
</tr>
<tr>
<td>Zhang et al. [67]</td>
<td>2008</td>
<td>China</td>
<td>4</td>
<td>4</td>
<td>21</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>2.1 ± 1.2 4.0 ± 1.4</td>
</tr>
<tr>
<td>Barath et al. [68]</td>
<td>2007</td>
<td>Hungary</td>
<td>4</td>
<td>4</td>
<td>72</td>
<td>CD25^{−}</td>
<td>Original</td>
<td>4.72 ± 2.3 5.0 ± 1.2</td>
</tr>
<tr>
<td>Lyssuk et al. [69]</td>
<td>2007</td>
<td>Russia</td>
<td>4</td>
<td>3</td>
<td>43</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>0.8 ± 0.4 1.8 ± 0.8</td>
</tr>
<tr>
<td>Lee et al. [70]</td>
<td>2006</td>
<td>Taiwan</td>
<td>4</td>
<td>3</td>
<td>27</td>
<td>CD25^{−}</td>
<td>Original</td>
<td>1.8 ± 0.8 4.9 ± 1.4</td>
</tr>
<tr>
<td>Suarez et al. [71]</td>
<td>2006</td>
<td>Spain</td>
<td>4</td>
<td>3</td>
<td>110</td>
<td>CD25^{−}</td>
<td>Calculated</td>
<td>6.1 ± 3.8 10.3 ± 3.9</td>
</tr>
<tr>
<td>Crispin et al. [72]</td>
<td>2003</td>
<td>Mexico</td>
<td>4</td>
<td>3</td>
<td>30</td>
<td>CD25^{+}</td>
<td>Calculated</td>
<td>18.6 ± 8.18 20.6 ± 5.9</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus. *Evidence level (EL) of each study was based on Oxford Center for Evidence-Based Medicine 2011. *Quality (Q) of each study was based on the Newcastle-Ottawa Quality Assessment Scale case.
when Tregs were defined as "CD25^{bright/high}CD127^{low/−}" cells \([-12.392, (-37.922, 12.138), p = 0.341]\) or "CD25^{high}CD127^{low/−}FOXp3^{+}\" cells \([-0.667, (-2.664, 1.331), p = 0.513]\) (Supplementary Figure 3 and Table 2).

As heterogeneity was apparent, we used a random effects model to prepare forest plots. We hypothesize that the significant heterogeneity might have been caused by differences in the experimental methods and clinical type and severity of disease among the different studies.

3.3. Disease Activity and the Proportion of Tregs in PB. To further assess the effect of disease activity, we analyzed 22 studies that reported the proportion of Tregs in active and inactive SLE patients, regardless of the Treg definitions used. We found a significant reduction in the proportion of Tregs in patients with active compared to inactive disease \([-0.520, (-0.976, -0.086), p = 0.019]\). The heterogeneity, as assessed by the \(I^2\) statistic, was 88.9% \((p < 0.001)\) (Figure 3). No publication bias was evident in the Egger test \((t = 0.52, p = 0.608)\).

4. Discussion

It is now widely accepted that the immune system includes Tregs that specialize in the maintenance of immune tolerance
CD25 alone is inadequate. In 2008, Han et al. [17] found that more precise markers are needed to identify Tregs. FOXP3, and CD127.

FOXP3 is a crucial regulator of Treg gene expression, being required for both Treg generation and survival [19]. Scurfy (Sf) mice with Treg abnormalities harbor a missense mutation in FOXP3 [6] and develop anti-dsDNA, anti-Smith, and antinuclear antibodies similar to those of SLE patients. Such FOXP3 mutant mice also exhibit multiorgan inflammation of systems usually involved in SLE [20]. However, when Tregs were defined as "FOXP3-positive" cells, the proportions of such cells did not differ between SLE patients and controls because the definitions of Tregs were complicated by the addition of CD25 status, giving "CD25-negative and FOXP3-positive" and "CD25 and FOXP3 double positive." This phenomenon may be explained by the findings of other studies indicating that the CD4+CD25+FOXP3+ T cells of SLE were dysfunctional compared to nTregs [25], Tr1 cells [26], Th1-like, Th2-like, or Th17-like Tregs [27], and so forth. One of the largest Treg subsets is nTregs, which are developed from the thymus and express CD4, CD25, and FOXP3 [24]. In contrast to nTregs, iTregs are generated in the periphery and induced to express FOXP3 in response to foreign antigens that are much intrinsically unstable in inflammatory compared to nTregs [25]. Interestingly, inflammatory conditions of stimulation can skew nTreg differentiation to Tr1 cells in active lupus [28]. Tr1 cells are...
another subset of CD4⁺ T cells in the absence of FOXP3 expression characterized by the ability to secrete IL-10 and inhibit T cell responses by disrupting the metabolic state of T effector cells [26]. Although there are important differences of these cells, there is no definitive protein markers that effectively distinguishes among all these Treg cell populations in vitro or in vivo. To date, it is still challenging to value the real status of above Treg subsets in patients with SLE.

The limitations of our work include the fact that we did not consider disease duration or treatment, as both the drugs used and disease staging were inconsistent; however, these factors might affect the proportion of Tregs in PB. Additionally, disease activity was scored differently among studies; some regarded active SLE to be present when the SLEDAI was ≥6, but others used different cutoffs; these differences may have influenced the results. Moreover, Tregs are usually evaluated in PB, in which tissue Treg cell status may fluctuate [3]. Also, information on Treg aberrations in lymphoid tissues or at sites of active disease, for example, the skin of patients with cutaneous lupus [29] or the kidneys of patients with active glomerulonephritis [30], was lacking.

5. Conclusion

In conclusion, we suggest that the reported variations of Treg status among SLE patients are attributable to inconsistent Treg identification; different markers are employed. Here, we analyzed the effects of the use of such markers on the reported proportion of Tregs. Our findings lend support to the idea that the Treg status of SLE patients is important, but we could not determine the best definition of Tregs. Further studies are needed on the definition and function of Tregs.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Sheng-Xiao Zhang and Xiao-Wen Ma contributed to the work equally and should be regarded as co-first authors.
Supplementary Materials

Supplementary Table 1: background of SLE patients in each study. Supplementary Table 2: characteristics of the included studies measuring disease activity in patients with SLE. Supplementary Figure 1: forest plot of subgroup analyses of the proportion of Tregs, defined in terms of CD25-positivity (alone), among CD4+ T cells in PB. (a) Standardized mean differences (SMDs) (the proportion of Tregs ["CD25-low/FoxP3-""] among CD4+ T cells in the PB of SLE patients minus that of control subjects) as estimated by meta-analysis. (b) SMDs when Tregs were defined as "CD25-bright" cells. (c) SMDs when Tregs were defined as "CD25-high" cells. Supplementary Figure 2: forest plots of subgroup analyses of the proportion of Tregs defined as FOXP3-positive among CD4+ T cells in PB. (a) SMDs (the proportion of Tregs ["CD25low/FoxP3-""] among CD4+ T cells in the PB of SLE patients minus that of control subjects) as estimated by meta-analysis. (b) SMDs when Tregs were defined as "FOXP3-" cells. (c) SMDs when Tregs were defined as "CD25low/FoxP3-" cells. (d) SMDs when Tregs were defined as "CD25high/FoxP3-" cells. Supplementary Figure 3: forest plots of the subgroup analyses of the proportion of Tregs that were CD127-negative among CD4+ T cells in PB. (a) SMDs (the proportion of Tregs ["CD25low/CD127-""] among CD4+ T cells in the PB of SLE patients minus that of control subjects) as estimated by meta-analysis. (b) SMDs when Tregs were defined as "CD25low/CD127-" cells. (c) SMDs when Tregs were defined as "CD25high/CD127-" cells. (d) SMDs when Tregs were defined as "CD25high/CD127bright" cells. Supplementary Figure 4: funnel plot for publication bias in an association analysis of Treg proportions in PB, regardless of the Treg definitions used, between systemic lupus erythematosus (SLE) patients and healthy controls. Supplementary Figure 5: funnel plot for publication bias in an association analysis of the proportion of Tregs, defined in terms of CD25-positivity (alone), among CD4+ T cells in PB. Supplementary Figure 6: funnel plot for publication bias in an association analysis of the proportion of Tregs defined as FOXP3-positive among CD4+ T cells in PB. Supplementary Figure 7: funnel plot for publication bias in an association analysis of the proportion of Tregs that were CD127-negative among PB CD4+ T cells. (Supplementary Materials)

References


[49] X. Pan, X. Yuan, Y. Zheng et al., "Increased CD45RA⁺FOXP3(low) regulatory T cells with impaired suppressive


