Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency characterized by thrombocytopenia, eczema, and variable degrees of impaired cellular and humoral immunity. Age-dependent T-cell lymphopenia has been described in WAS, however, the diversity of the T-cell compartment over time in these patients has not been characterized. We have used complementarity-determining region 3 (CDR3) size distribution analysis to assess T-cell receptor (TCR) Vβ repertoire in 13 patients with WAS. Diverse CDR3 size pattern was demonstrated in patients under 15 years of age regardless of the levels of WAS protein (WASP) expression. In contrast, older patients showed significantly higher skewing of TCRVβ repertoire as compared with healthy adults. We did not find correlation between clinical score and complexity of TCRVβ repertoire. These findings suggest that WASP deficiency does not limit thymic generation of a normal TCR and indicate that T-cell oligoclonality may contribute to the immunodeficiency in older patients with WAS. (Blood. 2005;106:3895-3897)

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Brief report
Analysis of T-cell repertoire diversity in Wiskott-Aldrich syndrome
Taizo Wada, Shepherd H. Schurman, Elizabeth K. Garabedian, Akihiro Yachie, and Fabio Candotti

Introduction
Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by thrombocytopenia, eczema, immunodeficiency, and increased risk for lymphoid malignancies and autoimmunity.1-2 Immunodeficiency in patients with WAS is heterogeneous and includes low serum immunoglobulin M (IgM), failure to produce antibodies to polysaccharide antigens, and in vitro deficits in T- and B-cell functions. The disease gene encodes the WAS protein (WASP), expressed in all nonerythroid hematopoietic cells.4 Although lack of WASP affects multiple cell lineages, the most prominent immunologic symptoms are attributable to defective T-cell function, which results from a combination of low T-lymphocyte numbers and intrinsic T-cell defects. Age-dependent T-cell lymphopenia has been described in patients with WAS, although T-cell numbers can be markedly low since early in age.4 Aside from immunophenotypic analysis of T-cell subsets,5 little is known about changes in the T-lymphocyte compartment of patients with WAS over time.

A sensitive method to study the T-cell receptor (TCR) repertoire is to determine size distribution of cDNA for the complementarity-determining region 3 (CDR3) of the TCRVβ region.6 The CDR3 is the hypervariable region that is generated by genetic rearrangements during T-cell maturation in the thymus. Study of the distribution of the CDR3 size allows us to determine clonal dominance or restriction within T-lymphocyte populations. Using this approach, we analyzed the entire TCRVβ repertoire in 13 patients with WAS and found significant skewing of TCRVβ usage in a subset of these subjects.

Study design
Patient samples
Blood samples were collected from 13 patients with WAS and 10 healthy adult volunteers after obtaining informed consent in accordance with National Human Genome Research Institute (NHGRI) institutional review board–approved clinical research protocols. Clinical diagnosis of WAS was confirmed by mutation analysis of the WAS gene and patients were assigned a clinical score based on disease severity using a previously described scoring system (Table 1).8 Flow cytometry analysis of WASP expression was performed as described.9

Analysis of CDR3 size distribution
Analysis of CDR3 size distribution was performed as described.9 To assess each individual profile as normal or skewed, we used a previously reported complexity scoring system.10,11 The number of TCRVβ subfamilies with skewed CDR3 size pattern, as shown by a complex score lower than 4, was determined for each subject.12 Analysis of differences among data groups was performed using the Student t test for unpaired samples. Values of P less than .05 were considered significant.

Results and discussion
WASP deficiency results in significant abnormalities of T-lymphocyte numbers and function. In patients with WAS, TCR stimulation with anti-CD3 antibodies results in defective cell proliferation, interleukin-2 production, up-regulation of CD69, actin polymerization, CD3 internalization, and lipid raft clustering.9,13-16 Similar T-cell defects are found in WASP knockout mice that also show defective antigen receptor capping and endocytosis after TCR stimulation.17,18 These defects have been attributed to the role of WASP in actin cytoskeleton remodeling after cell activation. However, the role of WASP in the T-cell lymphopenia characteristic of the disease remains unclear. In WASP knockout mice, Snapper et al17 reported normal thymic subsets despite diminished organ cellularity, whereas Zhang et al18 showed a block of...
progression of thymocytes from the double-negative (CD4⁻CD8⁻) to double-positive (CD4⁺CD8⁺) stage. Recent observations of low T-cell numbers in patients with WAS since infancy have been interpreted to support a role for WASP in lymphocyte maturation.⁶ On the other hand, evidence of enhanced spontaneous apoptosis in WAS lymphocytes has also been proposed as a possible mechanism for the pathogenesis of lymphopenia.¹⁹,²⁰ To contribute to the characterization of the T-lymphocyte compartment in patients with WAS, we investigated the diversity of 24 TCRVβ subfamilies by CD3R size distribution analysis.

In healthy controls, the majority of Vβ subfamilies exhibited a Gaussian curve with 6 peaks or more, reflecting polyclonal Vβ repertoire. Amplification of all 24 Vβ segments from each WAS patient’s sample allowed us to calculate the mean complexity score for all samples (5.04; range, 4.56-5.40) that did not considerably differ from that of healthy adults (5.06; range, 4.89-5.19; Figure 1A-B). However, the frequency of skewed TCRVβ subfamilies in samples from patients with WAS who are age 15 years or older was significantly higher than that observed in younger patients and samples from patients with WAS who are age 15 years or older was 1A-B). However, the frequency of skewed TCRVβ subfamilies is different from that of healthy adults (5.06; range, 4.89-5.19; Figure 1A). No correlation was observed between clonal pattern of TCRVβ repertoire and no evidence was found for preferential expansion of particular Vβ subfamilies in patients with a skewed pattern. Because none of the patients had clinical or laboratory evidence of acute infection at the time of sample collection, we conclude that these alterations reflect a stable state of the TCR repertoire in these patients.

In contrast to the older subjects with WAS, patients 14 years of age or younger showed a diverse distribution in the majority of their Vβ subfamilies, again regardless of the levels of WASP protein expression (Figure 1, Table 1). These findings suggest that WASP deficiency does not limit the generation of a wide repertoire of T lymphocytes early in life. Therefore, the skewed pattern of TCRVβ subfamilies in older patients with WAS does not seem to be the result of defective thymic generation of diverse TCRVβ-expressing populations. In support of this conclusion is the observation that patients with V(D)J recombination defects due to recombination activating gene (RAG) mutations show much more severe restriction of TCR repertoire with a mean complexity score of 1.73 (A.Y., unpublished observations, April 2005). The oligoclonal pattern of TCRVβ subfamilies usage in older patients with WAS likely develops over time due to the accumulation of antigen-specific T-cell clones. Although clonal T-cell expansion can be observed in healthy individuals with age,¹⁸ the increased oligoclonality of T lymphocytes in older patients with WAS may derive from chronic antigenic stimulation generated by the inability

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Score</th>
<th>Mutation</th>
<th>Gene location</th>
<th>Predicted effect</th>
<th>Protein expression</th>
<th>Lymphocytes, ( \times 10^9/L ) (n.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>10 mo</td>
<td>3*</td>
<td>71C &gt; T</td>
<td>Exon 1</td>
<td>Arg13Stop</td>
<td>Absent</td>
<td>4.5 (2.6-10.4)</td>
</tr>
<tr>
<td>W2</td>
<td>11 mo</td>
<td>2*</td>
<td>290C &gt; T</td>
<td>Exon 2</td>
<td>Arg96Cys</td>
<td>ND</td>
<td>4.8 (2.6-10.4)</td>
</tr>
<tr>
<td>W3</td>
<td>12 mo</td>
<td>2*</td>
<td>6-bp ins nt 434</td>
<td>Exon 4</td>
<td>Asp63Ins</td>
<td>Absent</td>
<td>4.4 (2.6-10.4)</td>
</tr>
<tr>
<td>W4</td>
<td>15 mo</td>
<td>3*</td>
<td>G &gt; A (+ 1)</td>
<td>Intron 8</td>
<td>fs, stop aa246</td>
<td>Absent</td>
<td>2.4 (2.6-10.4)</td>
</tr>
<tr>
<td>W5</td>
<td>7 y</td>
<td>1</td>
<td>1487G &gt; A</td>
<td>Exon 11</td>
<td>Asp85Asn</td>
<td>Reduced</td>
<td>3.3 (1.1-5.9)</td>
</tr>
<tr>
<td>W6</td>
<td>14 y</td>
<td>2</td>
<td>291G &gt; A</td>
<td>Exon 2</td>
<td>Arg86His</td>
<td>Reduced</td>
<td>1.5 (1.0-5.3)</td>
</tr>
<tr>
<td>W7</td>
<td>15 y</td>
<td>2</td>
<td>291G &gt; A</td>
<td>Exon 2</td>
<td>Arg86His</td>
<td>Reduced</td>
<td>1.3 (1.0-5.3)</td>
</tr>
<tr>
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<td>15 y</td>
<td>5</td>
<td>953A &gt; G</td>
<td>Exon 9</td>
<td>Met307Val</td>
<td>Reduced</td>
<td>2.4 (1.0-5.3)</td>
</tr>
<tr>
<td>W9</td>
<td>15 y</td>
<td>3</td>
<td>290C &gt; T</td>
<td>Exon 2</td>
<td>Arg96Cys</td>
<td>Reduced</td>
<td>3.5 (1.0-5.3)</td>
</tr>
<tr>
<td>W10</td>
<td>18 y</td>
<td>3</td>
<td>290C &gt; T</td>
<td>Exon 2</td>
<td>Arg96Cys</td>
<td>Reduced</td>
<td>1.6 (1.0-2.8)</td>
</tr>
<tr>
<td>W11</td>
<td>19 y</td>
<td>5</td>
<td>150T &gt; C</td>
<td>Exon 1</td>
<td>Leu39Pro</td>
<td>Absent</td>
<td>0.9 (1.0-2.8)</td>
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<tr>
<td>W12</td>
<td>22 y</td>
<td>1</td>
<td>389G &gt; A</td>
<td>Exon 3</td>
<td>Gly119Arg</td>
<td>Reduced</td>
<td>2.1 (1.0-2.8)</td>
</tr>
<tr>
<td>W13</td>
<td>36 y</td>
<td>2</td>
<td>995C &gt; T</td>
<td>Exon 10</td>
<td>Arg321Stop</td>
<td>ND</td>
<td>1.1 (1.0-2.8)</td>
</tr>
</tbody>
</table>

n.v. indicates normal values; ND, not determined.
*The score compatible with the clinical presentation of the patient is listed, although the full picture of the disease may have not yet developed due to the patient’s young age.

Figure 1. Analysis of T-cell repertoire. (A) CDR3 size distribution of TCRVβ subfamilies. Each TCRVβ fragment was amplified from cDNA with 1 of 24 Vβ-specific primers. The size distribution of polymerase chain reaction (PCR) products was determined by an automated sequencer and GeneScan software. (B) Complexity scores of TCRVβ. Complexity scores were generated for each TCRVβ from the CDR3 size distribution analysis.⁷ (C) Frequency of skewed TCRVβ repertoire. Shown are the mean (± standard deviation [SD]) numbers of skewed TCRVβ obtained from the control and patient groups. *P < .05.
to completely eradicate infectious agents or, in some cases, be sustained by autoantigens.2 On the other hand, oligoclonality in WAS could be the result of homeostasis-driven T-cell proliferation in response to lymphopenia caused by increased apoptosis19,20 or, possibly, accelerated reduction of thymic output. The latter possibility could be addressed by analysis of TCR rearrangement excision circles.21 These and further studies assessing TCR repertoire in isolated T-cell subsets (eg, CD4+, CD8+) will be useful to further define the origin of clonal T-cell dominance in WAS.

In summary, our results demonstrate a significant skewing of CDR3 size distribution in T lymphocytes from older patients with WAS, add to the knowledge of T-cell defects in WAS, and point to an additional component of the immune dysregulation in this disease.

Acknowledgment

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References

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