We previously reported on a 43-year-old patient with Wiskott-Aldrich syndrome (WAS) who experienced progressive clinical improvement and revertant T-cell mosaicism. Deletion of the disease-causing 6-bp insertion was hypothesized to have occurred by DNA polymerase slippage. We now describe 2 additional patients from the same family who also had revertant T lymphocytes that showed selective in vivo advantage. Somatic mosaicism was demonstrated on leukocytes cryopreserved in the first patient when he was 22 years old, 11 years before his death from kidney failure. The second patient is now 16 years old, has a moderate clinical phenotype, and developed revertant cells after the age of 14 years. These results support DNA polymerase slippage as a common underlying mechanism, and they indicate that T-cell mosaicism may have different clinical effects in WAS. (Blood. 2004;104:1270-1272)

**Analysis of WASP gene mutations and expression**

Analysis of the WASP gene mutations and FACS analysis of WASP expression was performed as described. Detection of WASP exon 4 mutated and revertant sequences from genomic DNA of fresh or cryopreserved PBMCs was performed after polymerase chain reaction (PCR) amplification, as described, followed by subcloning of PCR products (Topo TA; Invitrogen, Carlsbad, CA) and sequencing of isolated clones.
Recently described revertant mosaicism in patient III-1 (Figure 1A). Because the ACGAGG insertion followed a tandem repeat of the same 6-bp sequence, we hypothesized that both the original mutation and the reversion were caused by a DNA polymerase slippage facilitated by the specific structural characteristics of the neighboring DNA. Accordingly, other affected members from the same family would be expected to be prone to similar slippage events and consequent somatic mosaicism. Therefore, we studied WASP expression in fresh PBMCs from patients III-1, III-2, III-6, and IV-1 and frozen PBMCs from patient III-2 at the age of 22 years.

As in patient III-1, WASP-expressing cells were clearly detectable only among T lymphocytes of patients III-2 and III-6, CD4+ and CD8+ T-cell subsets contained revertant cells (Table 1), whereas no WASP-expressing cells were detectable within B and natural killer (NK) cells. These findings suggest that the revertant event occurred at the level of a T-cell progenitor before CD4/CD8 commitment. Alternatively, the reversion might have occurred in a multipotent hematopoietic progenitor, but it manifested itself only within T cells in which WASP-expressing cells have a selective advantage. Evidence of selective advantage of the revertant T lymphocytes was also found in patients III-2 and III-6, whose percentage of WASP-expressing cells among CD45RO-memory T cells was markedly higher than in CD45RO-naïve T lymphocytes (Table 1).

Revertant somatic mosaicism has been described in an increasing number of primary immunodeficiencies and has resulted in progressively improving or mild clinical phenotype in patients with adenosine deaminase deficiency, X-linked severe combined immunodeficiency, and WAS (patient III-1). In the latter, revertant cells comprised approximately 80% of the patient’s lymphocytes and CD3+ T cells (Table 1). In 3 other WAS patients with levels of revertant lymphocytes ranging between 10% and 15%, clinical improvement could not be demonstrated.

In one of these patients, revertant cells were clearly detected only among CD8+ fresh T cells (although revertant CD4+ T cells could be demonstrated after immortalization), whereas the other 2 patients carried approximately 25% to 37% of revertant CD3+ T cells, with higher proportions of revertant cells in CD4+ T cells than in the CD8+ subset. All 3 patients had clinical scores of 5 when revertant cells were described. Information is limited regarding 5 additional patients with revertant WAS. Previous studies, therefore,

### Table 1. Immunologic features and WASP expression in lymphocyte subsets

<table>
<thead>
<tr>
<th></th>
<th>III-1</th>
<th>III-2*</th>
<th>III-6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute blood counts, × 10^9/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells</td>
<td>13.8</td>
<td>11.2</td>
<td>11.1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>6.19</td>
<td>2.24</td>
<td>3.4</td>
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<tr>
<td>CD3+ T cells</td>
<td>5.8</td>
<td>1.65</td>
<td>1.29</td>
</tr>
<tr>
<td><strong>WASP+ cells in lymphocyte subsets, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>79.5</td>
<td>56.7</td>
<td>11.7</td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>79.6</td>
<td>79.5</td>
<td>27.6</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>85.9</td>
<td>60.3</td>
<td>1.9</td>
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<tr>
<td>CD8+ T cells</td>
<td>69.7</td>
<td>79.4</td>
<td>64.3</td>
</tr>
<tr>
<td>CD45RO+CD3+ T cells</td>
<td>42.0</td>
<td>65.4</td>
<td>18.9</td>
</tr>
<tr>
<td>CD45RO+CD3+ T cells</td>
<td>86.0</td>
<td>87.3</td>
<td>43.0</td>
</tr>
<tr>
<td>CD20+ B cells</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>CD56+ NK cells</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND indicates not detected; and NA, not available.

Immunophenotyping and flow cytometric analysis of WASP were performed on frozen samples.
suggested that high levels of revertant T cells may be needed to elicit clinical effects in WAS.

Regarding the patients of our study, the overall moderate phenotype of patient III-6 does not allow us to draw clear conclusions about the effects of revertant cells. In patient III-2, the severe complications of the disease were apparently not mitigated by the presence of approximately 80% WASP-expressing T lymphocytes. One possible explanation is that these cells might have appeared and accumulated in this patient after multiple and irreversible organ damage had developed. Results from previous studies combined with our current observations indicate that despite consistent evidence of an in vivo selective advantage of revertant, WASP-expressing T lymphocytes, the potential clinical consequences of T-cell mosaicism in WAS remain difficult to predict.

Siblings with revertant mosaicism11,19,21 have been reported, but, to our knowledge, 3 patients with revertant disease in a single kindred is unprecedented. The finding of the same true back mutation in all 3 patients with mosaicism strongly suggests a common underlying mechanism that likely involves DNA polymerase slippage.

Finally, in vivo reversion appears to be a phenomenon of relatively high incidence in WAS patients. It seems reasonable to assume that the rate of reversion is similar in WAS as it is in other disorders. If this is so, the high incidence of reversion events in WAS could be attributed to the increased survival of WAS patients, providing a favorable biologic environment to allow revertant cells to accumulate to levels that reach the detection threshold. Alternatively, it is tempting to speculate that increased genomic instability in WAS is responsible for a higher rate of reversion events.

Acknowledgments

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References

Multiple patients with revertant mosaicism in a single Wiskott-Aldrich syndrome family

Taizo Wada, Shepherd H. Schurman, G. Jayashree Jagadeesh, Elizabeth K. Garabedian, David L. Nelson and Fabio Candotti