Brief report

Differential contribution of Wiskott-Aldrich syndrome protein to selective advantage in T- and B-cell lineages

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Somatic mosaicism because of in vivo reversion has been recently reported in a small number of patients affected with Wiskott-Aldrich syndrome (WAS). Flow cytometry analysis of WAS protein (WASP) expression has shown that these patients carried revertant cells only among T lymphocytes. Here, we have used high-resolution capillary electrophoresis to analyze genomic DNA from highly purified cells of one of these patients and detected revertant sequences also within the B-cell fraction. The demonstration of revertant cells among both T and B lymphocytes in this patient is consistent with the reversion event having occurred in a common lymphoid progenitor. However, although WASP-expressing T cells showed selective advantage and were readily detectable in the periphery of the mosaic patient, revertant B lymphocytes remained below the detection threshold of flow cytometry. These findings suggest that, contrary to T cells, differentiation and survival of B lymphocytes is minimally dependent on WASP.

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Introduction

Revertant mosaicism is a rare phenomenon that is increasingly being detected in human diseases, including the Wiskott-Aldrich syndrome (WAS [MIM 300100]). Interestingly, in all cases of WAS with somatic mosaicism reported to date, revertant cells were observed only among T lymphocytes. Although it is conceivable that in all cases the reversion events occurred at the level of T-lymphocyte precursors, the possibility also exists that more primitive progenitors were involved and that revertant cells only accumulated among T lymphocytes because WAS protein (WASP) expression in other hematopoietic cells may not engender equal selective advantage. Very recently, we observed an additional family with WAS in which 2 brothers presented with somatic mosaicism because of the same second-site mutation. Similarly to previous reports, both patients demonstrated revertant, WASP-expressing cells only among T lymphocytes. Molecular evidence of revertant sequences within DNA isolated from B lymphocytes could not be confirmed because of the possible presence of contaminating revertant T lymphocytes within the B-cell sample. Here, we have extended our molecular analysis of B lymphocytes from 1 of the 2 mosaic subjects. Using high-resolution capillary electrophoresis on highly purified cells we have demonstrated the presence of revertant B lymphocytes in this patient.

Study design

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were obtained in accordance with a National Human Genome Research Institute institutional review board (IRB)-approved protocol and upon informed consent from WAS patient II-1 and were depleted of CD3+ T lymphocytes with the use of magnetic beads as described. CD3+ cells were then stained with anti-CD20, anti–T-cell receptor (TCR)αβ, and anti-TCRγδ by isolation of highly purified CD20+/TCRαβ−/TCRγδ− cells with 2 rounds of cell sorting with the use of FACSVantage (BD Biosciences, San Jose, CA).

GeneScan analysis and sequencing

The DNA equivalent of 50 000 CD20+/TCRαβ−/TCRγδ− sorted B lymphocytes and CD3+ T cells was subjected to GeneScan analysis (Perkin-Elmer Applied Biosystems, Foster City, CA) for the presence of the original single nucleotide insertion (1305insG) and the second-site 19-base pair (bp) deletion (Δ19bp) as described. Additional GeneScan analysis and direct sequencing of the polymerase chain reaction (PCR) products from B cells was performed as described after DruII digestion and a second round of amplification.

Results and discussion

Our patient with WAS is known to carry revertant T lymphocytes because of the presence of the Δ19bp mutation, a 19-bp deletion that removes the disease-causing guanine insertion (1305insG) and restores the WASP reading frame and protein function (Figure 1A). In the original description, WASP-expressing cells were only detected among T lymphocytes by fluorescence activated cell sorting (FACS) analysis, and the genomic WASP fragment generated by the Δ19bp mutation was identified in T cells but not in granulocytes, monocytes, or natural killer (NK) cells. A minor fragment of the size compatible with the Δ19bp mutation was
observed in DNA extracted from sorted B lymphocytes but could not definitively be attributed to the presence of revertant B cells because of the possibility that it originated from contaminating T lymphocytes. To obtain a population of B lymphocytes free from contaminating T cells, CD3-depleted CD20+/TCRαβ-/TCRγδ- cells were isolated by FACS and demonstrated free from CD3+ T cells (data not shown). As expected, sorted B lymphocytes lacked expression of CD3ε mRNA (data not shown). The DNA equivalent of 50,000 CD20+/TCRαβ-/TCRγδ- sorted B lymphocytes and immunomagnetically isolated CD3+ T cells was amplified with a primer set flanking the mutated WASP exon 10 sequence, and the PCR product was subjected to GeneScan analysis for the presence of fragments representing the 1305insG and Δ19bp mutations. A peak of the approximate size of 190 nucleotides (n’s) was detected in both samples and indicated the presence of the Δ19bp mutation (Figure 1B, peak Δ19). To demonstrate that the relative PCR fragment derived from the revertant sequence, the B-cell amplification product was digested with the DrAlII restriction enzyme that recognizes the GGGGCCT sequence located within the 19bp deletion (Figure 1A). Consequently, the DrAlII digestion of the PCR product was digested and subjected to direct sequencing, which confirmed the presence of the Δ19bp deletion observed in revertant cells (Figure 1A).

These results demonstrated the presence of the Δ19bp deletion in both T and B lymphocytes of our WAS patient with somatic mosaicism and are compatible with the occurrence of the second-site mutation at the level of a multipotent hematopoietic progenitor, such as the common lymphoid progenitor (CLP). Somatic mosaicism because of in vivo reversion that likely occurred in a multipotent hematopoietic progenitor has been described for adenosine deaminase deficiency and Fanconi anemia, and a similar event can, therefore, be responsible for the presence of the Δ19bp mutation in both T and B lymphocytes in our patient with WAS. However, the possibility that suppressing mutations occurred independently in the T- and B-cell lineages cannot be ruled out and could be explained by an elevated spontaneous mutation rate of the genomic sequence surrounding the original 1305insG mutation because of increased tendency for hairpin formation.

Whatever causal mechanism underlies the presence of the Δ19bp deletion in both T and B lymphocytes, it is important to note that the restoration of a functional form WASP had profound different effects on T and B cells. Revertant T cells were readily detectable by FACS, accumulated over time, and showed in vivo selective advantage. On the contrary, no WASP-expressing B lymphocytes could be detected by FACS analysis in the peripheral blood of either brother, although the presence of the Δ19bp second-site mutation could clearly be demonstrated at the molecular level (Figure 1). The striking difference in accumulation of revertant T and B cells could be simply due to a shorter life span of B lymphocytes compared with T cells. However, these findings are also compatible with the hypothesis that WASP-expressing
B lymphocytes have minimal selective advantage over the WASP-negative counterparts.

The recent description of a female patient presenting with WAS symptoms because of a failure to inactivate the X chromosome carrying the mutated WASP allele has provided evidence that T but not B lymphocytes expressing WASP were selected in vivo, thus further indicating the lack of selective pressure for B cells expressing WASP. These data and our results support a reduced role for WASP in B-cell differentiation, proliferation, and survival, which is in line with the observations in WASP knock-out mice that show unchanged or only minimal B-cell defects.

The recognition of differential selective advantage conferred by WASP expression to T and B (and possibly other hematopoietic) lineages will provide important insights into the biology of the disease, help explain the occurrence of mixed chimerism in patients with WAS after allogeneic bone marrow transplantation, and offer valuable perspectives on the possible outcomes of gene therapy for WAS.

References

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