

Case Study

B-Cell Prolymphocytic Leukemia Carrying t(8;14)(q24;q32), Associated with Both Autoimmune Hemolytic Anemia and Pure Red Cell Aplasia

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An 80-year-old man was referred to our department because of lymphocytosis. His white cell count was $17.1 \times 10^3/\mu\text{L}$, with 64% prolymphocytes. He did not exhibit splenomegaly or lymphadenopathy. Prolymphocytes were CD5⁺, CD10⁻, CD19⁺, CD20⁺, CD21^{+weak}, CD22⁺, CD23⁻, and HLA-DR⁺, and expressed $\mu\delta/\lambda$ cell-surface immunoglobulins. G-banding and fluorescence *in situ* hybridization using *c-MYC* and immunoglobulin heavy-chain (*IgH*) gene probe revealed that leukemia cells carried the t(8;14)(q24;q32)/*c-MYC-IgH* fusion gene, and breakage and reunion occurred within the non-coding region of *c-MYC* exon 1 as well as the α switch region of *IgH*. Nine months after the initial presentation, the patient's hemoglobin level fell to 5.7 g/dL. Coombs' test was positive and marked hypoplasia of erythroid precursors was detected in his bone marrow. The patient was treated with prednisolone followed by 4 weekly doses of rituximab, which led to resolution of the anemia and complete response of the underlying leukemia. The role of t(8;14)(q24;q32)/*c-MYC-IgH* in the pathogenesis of B-cell prolymphocytic leukemia (B-PLL) may not be identical to that in aggressive lymphoid neoplasms, and, in the present case, autoantibodies targeting both mature red cells and erythroid precursors may have been concurrently produced in the setting of B-PLL. [*J Clin Exp Hematop* 54(3) : 219-224, 2014]

Keywords: B-cell prolymphocytic leukemia, t(8;14)(q24;q32)/*c-MYC-IgH*, autoimmune hemolytic anemia, pure red cell aplasia, rituximab

INTRODUCTION

B-cell prolymphocytic leukemia (B-PLL) is a rare lymphoid neoplasm that is characterized by the proliferation of prolymphocytes with the B-cell phenotype in the peripheral blood, bone marrow, and spleen.¹ Most patients present with a marked increase in white cell counts and splenomegaly, and respond poorly to the standard treatments for B-cell chronic lymphocytic leukemia (B-CLL).¹⁻³ In addition to its morphological and clinical features, the differential diagnosis of B-PLL from other B-cell leukemias/lymphomas requires immunophenotypic and cytogenetic studies. In contrast to B-CLL cells, B-PLL cells strongly express surface immunoglobulin ($\mu \pm \delta$) and pan-B markers (CD19, CD20, CD22, and CD79a), and the positivity of CD5 and CD23 was previously shown to be 20-30% and 10-20%, respectively.¹ The presence of t(11;

14)(q13;q32) and its molecular equivalent indicates the leukemic manifestation of mantle cell lymphoma instead of B-PLL.¹⁻³

Immune-mediated anemia is often associated with lymphoid neoplasms at variable frequencies.⁴ Autoimmune hemolytic anemia (AIHA) is the most common autoimmune complication of B-CLL, with a frequency of 5 to 10%.⁵ On the other hand, sporadic case reports and small case series of lymphoid neoplasms associated with pure red cell aplasia (PRCA) are found in the literature.^{4,6,7} The mechanisms responsible for the two types of anemia may be attributable to the production of autoantibodies against red cells or their precursors by neoplastic cells themselves or polyclonal B cells stimulated by the underlying neoplasm.

We here describe a case of B-PLL, the leukemia cells of which carried t(8;14)(q24;q32). This disease was associated with progressive anemia and finally with the aplastic crisis of red cell precursors in the bone marrow. The morphology, immunophenotype, and cytogenetic abnormalities of leukemia cells as well as their response to treatments with prednisolone and rituximab are described.

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CASE REPORT

An 80-year-old man was referred to our department because of lymphocytosis for a duration of over two years. He was asymptomatic with no hepatosplenomegaly or superficial lymphadenopathy. His hemoglobin level was 13.3 g/dL, platelet count $175 \times 10^3/\mu\text{L}$, and white cell count $17.1 \times 10^3/\mu\text{L}$. His white cells included 64% prolymphocytes with moderately condensed nuclear chromatin, prominent nucleoli, and a faintly basophilic cytoplasm (Fig. 1A). His blood chemistry was unremarkable, except for a lactate dehydrogenase level of 315 IU/L. His soluble interleukin-2 receptor level was 2,480 U/mL (normal range, 135-483 U/mL).

A bone marrow aspirate smear showed 12.0% erythroid lineage cells, 13.8% myeloid lineage cells, and 69.1% prolymphocytes (Fig. 1B); megakaryocytes appeared normal. Prolymphocytes were CD5⁺, CD10⁻, CD19⁺, CD20⁺, CD21^{+weak}, CD22⁺, CD23⁻, and HLA-DR⁺, as determined by two-color flow cytometry (Fig. 2). These cells expressed the μ and δ heavy chains in association with the λ light chain on their surfaces (Fig. 2). The DNA index compared with that of normal diploid cells was 1.00. A bone marrow core biopsy sample was normocellular with interstitial infiltrates of intermediate-sized lymphoid cells and normal trilineage hematopoiesis. Immunohistochemistry confirmed that the infiltrated cells were positive for CD5 and CD20, and negative for CD3 and cyclin D1. *In situ* hybridization for Epstein-Barr virus-encoded RNA was negative.

Cytogenetic and molecular analyses

G-banding of metaphase spreads obtained from the bone marrow revealed two types of aneuploid cell, both of which had a 14q + marker chromosome. We next performed fluorescence *in situ* hybridization (FISH) of the bone marrow smear slide using the *c-MYC* and immunoglobulin heavy-

chain (*IgH*) gene dual fusion probe (Vysis IGH/MYC/CEP8 Tri-Color DF FISH Probe Kit, Abbott Laboratories), and found that 54.8% of the nuclei contained two yellow signals indicative of the *c-MYC-IgH* fusion gene (Fig. 3A). We then applied the probe to the chromosome preparation and identified the der(8) and der(14) chromosomes involved in t(8;14)(q24;q32) (Fig. 3B). The other *c-MYC* signal was localized on the end of the marker chromosome, which was determined to be der(3)t(3;8)(p13;q13) (Fig. 3B), or on normal chromosome 8. The complete karyotype of leukemia cells according to the ISCN⁸ was 47,XY,+der(3)t(3;8)(p13;q13),-8,t(8;14)(q24;q32),+18[5]/48,XY,+add(3)(p13),t(8;14)(q24;q32),+18[2]/46,XY[4] (Fig. 4).

To confirm t(8;14)(q24;q32)/*c-MYC-IgH*, high-molecular-weight genomic DNA extracted from the peripheral blood was subjected to a long-distance polymerase chain reaction (LD-PCR) using primers designed for *c-MYC* exon 2 as well as the 4 constant region genes of *IgH*.⁹ Fig. 5A shows the results of LD-PCR with a combination of the *c-MYC* and α constant gene primers. The LD-PCR product, which was 5.4 kb in size, was cloned into the plasmid (pGEM[®]-T Easy, Promega), and three independent cloned DNAs were sequenced using an ABI 310 automated sequencer (Applied Biosystems) in order to avoid PCR artifacts. The nucleotide sequences of the regions of interest were analyzed *via* the University of California Santa Cruz Genome Bioinformatics database using BLAT (genome.ucsc.edu/cgi-bin/hgBlat/) in order to determine the sequence of the *c-MYC-IgH* junction, in which breakage and reunion occurred within the non-coding region of *c-MYC* exon 1 and the α switch region of *IgH* (Fig. 5B).

Treatment course

Although the patient's condition had been good for several months, his hemoglobin level gradually fell to 9.1 g/dL

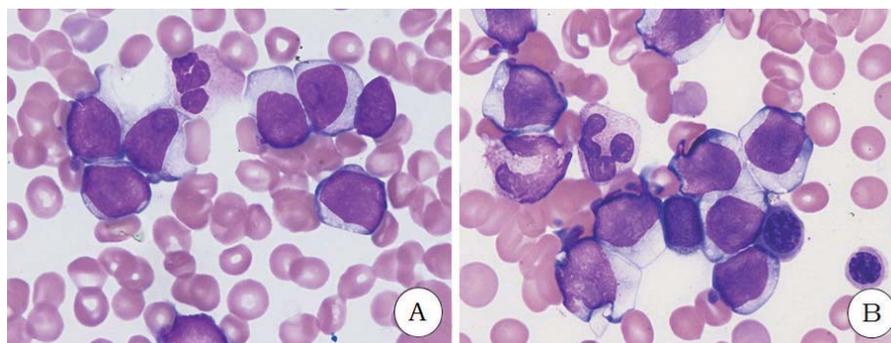


Fig. 1. Appearance of leukemia cells in the peripheral blood (1A) and bone marrow (1B). These cells were characterized by moderately condensed nuclear chromatin, prominent nucleoli, and a faintly basophilic cytoplasm. Wright staining; original magnification, $\times 100$ objective.

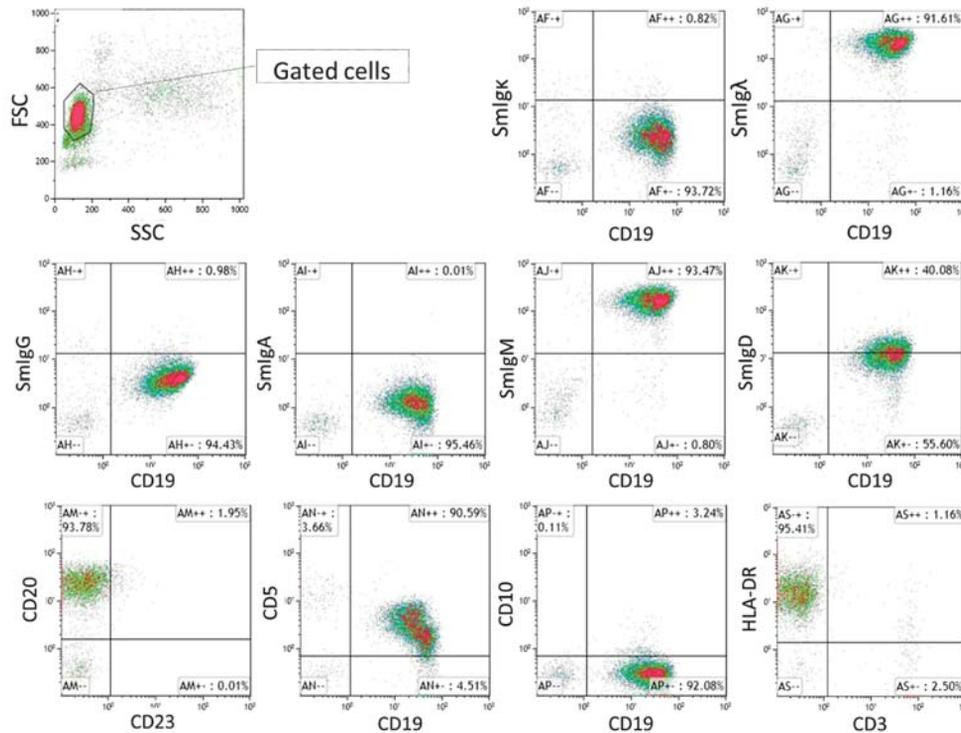


Fig. 2. Flow cytometry of leukemia cells. Gated cells on the SSC-FSC scattergram were separated by the expression of the antigens indicated. These cells were positive for CD5, CD19, CD20, and HLA-DR, but were negative for CD10 and CD23. Cell surface immunoglobulins were the μ and δ heavy and λ light chains.

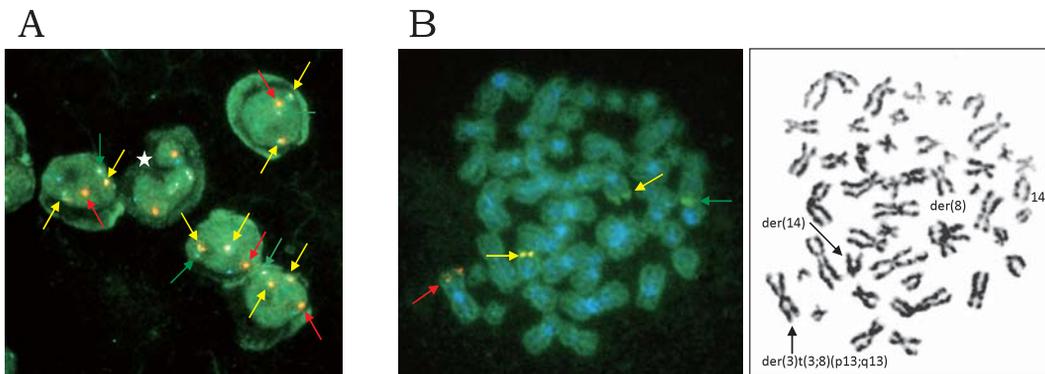


Fig. 3. Fluorescence *in situ* hybridization (FISH) showing the *c-MYC-IgH* fusion gene and t(8;14)(q24; q32). (3A) FISH of the bone marrow smear slide. Round nuclei corresponding to prolymphocytes contain one green (*IgH*), one red (*c-MYC*), and two yellow (*c-MYC-IgH* fusion) signals, as indicated by the arrows of each color. A lobulated nucleus corresponding to myeloid cells carries two green and two red signals (asterisk). The blue signals represent the centromere region of chromosome 8 (CEP8). (3B) Metaphase FISH showing the fusion signals on the der(8) and der(14) chromosomes (yellow arrows). One *IgH* signal was localized on normal chromosome 14 (green arrow) and one *c-MYC* signal was on der(3)t(3;8)(p13;q13) (red arrow). The G-banded picture is shown on the right as a reference. The karyotype of this metaphase chromosome was 47,XY,+der(3)t(3;8)(p13;q13),-8,t(8;14)(q24;q32),+18.

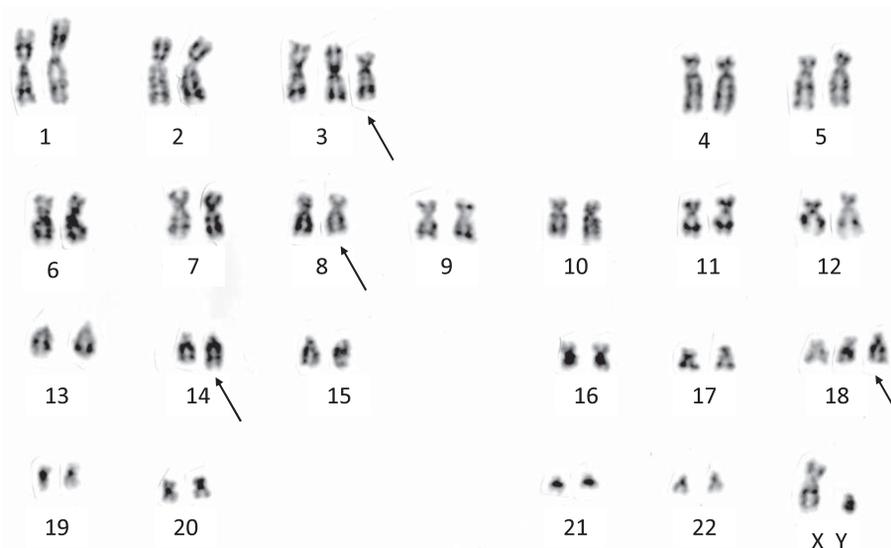


Fig. 4. The G-banded karyotype obtained from the bone marrow. The karyotype was 48,XY,+add(3)(p13),t(8;14)(q24;q32),+18. The arrows indicate add(3)(p13), der(8)t(8;14)(q24;q32), and der(14)t(8;14)(q24;q32) chromosomes and +18.

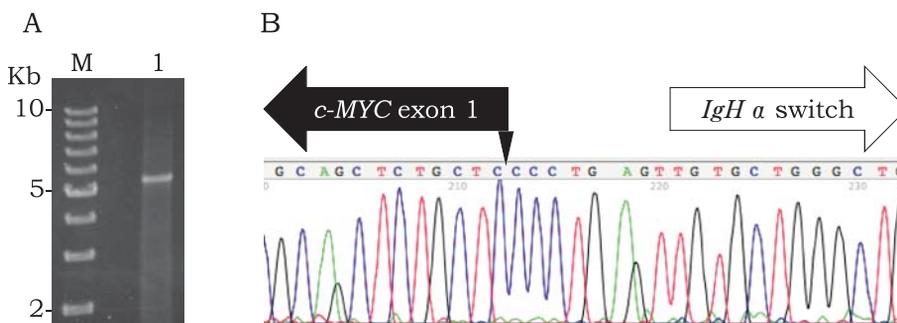


Fig. 5. Long-distance polymerase chain reaction (LD-PCR) showing the *c-MYC-IgH* fusion gene. (**5A**) Ethidium bromide-stained gel electrophoresis of LD-PCR products. Lane M, 1-kb ladder as a molecular weight marker; and lane 1, peripheral blood of this case. The primers were: MYC/04, 5'-ACAGTCCTGGATGATGATGTTTTGATGAAGGTCT-3'; and IGHA/01, 5'-TTCGTGTAGTGCTTCACGTGGCATGTCACGGACTT-3'.⁹ (**5B**) Nucleotide sequences encompassing the t(8;14)(q24;q32)/*c-MYC-IgH* junction. A closed arrowhead indicates the breakpoint within *c-MYC* exon 1, which was located 320 bp upstream of the exon 1-intron 1 boundary of *c-MYC*. An 8-bp segment of unknown origin was inserted at the junction.

(Fig. 6). Both direct and indirect Coombs' tests were positive and his haptoglobin value was 1.3 mg/dL (normal range, 9-40 mg/dL). Nine months after his initial presentation, he was admitted to our department because of increasing dyspnea on exertion. On examination, he appeared to be markedly anemic, but did not exhibit hepatosplenomegaly or lymphadenopathy. Laboratory tests revealed the following: hemoglobin level, 5.4 g/dL; white cell count, $16.7 \times 10^3/\mu\text{L}$, including 63.5% prolymphocytes; and platelet count, $202 \times 10^3/\mu\text{L}$. The proportion of reticulocytes was 0.1% of his red blood cells ($3,660/\mu\text{L}$); serum iron level, 251 $\mu\text{g/dL}$; ferritin, 572

ng/mL; lactate dehydrogenase, 382 IU/L; and total bilirubin, 1.4 mg/dL. Marked erythroid hypoplasia was observed in the bone marrow and comprised 0.6% of the nucleated cells, while myeloid lineage cells and megakaryocytes were within adequate ranges. The extent of prolymphocyte infiltration was not significantly different from that in the initial examination. Serological tests for parvovirus B19 IgM and the DNA of this virus were negative.

The patient was initially treated with prednisolone, which led to a burst of reticulocytes and the deterioration of peripheral prolymphocytosis (Fig. 6). He then received 4 weekly

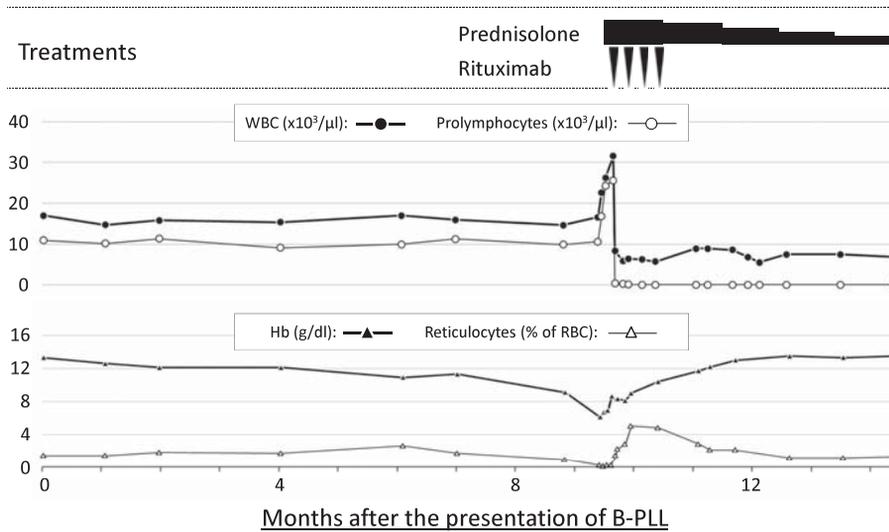


Fig. 6. Clinical course and response to treatment. The treatment consisted of prednisolone, which was started at a dose of 60 mg/day and tapered as illustrated, and weekly doses of 375 mg/m² rituximab. The counts of white cells (WBC) and prolymphocytes in the peripheral blood, level of hemoglobin (Hb), and percentage of reticulocytes are shown.

doses of 375 mg/m² rituximab; we divided the first dose into 50 mg/m² on day 1 and 325 mg/m² on day 3 to prevent an infusion reaction. As shown in Fig. 6, prolymphocytes quickly disappeared from the peripheral blood and his hemoglobin level steadily increased. Other laboratory data related to AIHA and PRCA were within normal ranges. His condition is currently good, having achieved complete remission (CR) of the underlying leukemia 40 months after his first referral to our department.

DISCUSSION

We herein describe a case of B-PLL carrying t(8;14)(q24;q32)/*c-MYC-IgH*, which was determined by both G-banding and interphase/metaphase FISH. LD-PCR and nucleotide sequencing analyses confirmed the *c-MYC-IgH* rearrangement, in which the positions of the breakpoints on both *c-MYC* and *IgH* were similar to those of sporadic-type Burkitt lymphoma.⁹ The patient did not exhibit the typical features of B-PLL; namely, splenomegaly was absent and only a modest increase was observed in the peripheral white cell count. Nevertheless, the characteristic morphology of leukemic cells and strong expression of surface immunoglobulins and B-cell antigens favored the diagnosis of B-PLL.

Only a few studies have involved cytogenetic analyses of B-PLL due to the rarity of this disease and difficulties associated with examining metaphase chromosomes.³ Nevertheless, cases of B-PLL that carry t(8;14)(q24;q32) have been sporadically reported in the literature.¹⁰⁻¹⁴ As t(8;14)(q24;q32)/*c-MYC-IgH* leads to deregulated expression of the

c-MYC oncoprotein, which is a transcriptional factor that activates genes involved in cell proliferation and growth, tumors carrying the translocation generally exhibit aggressive histopathological and clinical behaviors.¹⁵ In B-CLL, the presence of t(8;14)(q24;q32) indicates cytological and clinical transformations to an aggressive disease as well as a poor clinical outcome.¹⁶ On the other hand, a previous study described 9 cases of B-PLL that carried the *c-MYC* translocation determined by FISH; the median time to treatment was as short as 1.5 months, 5 of the 9 cases achieved CR or a partial response by the initial treatment, and the median overall survival from the clinical diagnosis was extended to 110 months.¹⁷ In the present case, B-PLL itself exhibited indolent clinical behavior and responded well to rituximab, which led to CR. Thus, the role of t(8;14)(q24;q32)/*c-MYC-IgH* in the pathogenesis of *de novo* B-PLL may not be identical to that in aggressive lymphoid neoplasms. A very speculative mechanism for this is that high-level expression of *c-MYC* targets genes and collaborating genetic mutations,^{18,19} both of which effectively separate Burkitt lymphoma from diffuse large B-cell lymphoma and are likely to correlate with the aggressive behavior, which may not be the case in B-PLL carrying t(8;14)(q24;q32)/*c-MYC-IgH*.

The patient developed progressive anemia and finally the aplastic crisis of erythroid precursors. Laboratory tests and a bone marrow examination suggested that the anemia resulted from mechanisms mediating both AIHA and PRCA; that is, positive antiglobulin tests and a low haptoglobin level indicated the former, while reticulocytopenia in the peripheral blood and restricted erythroid hypoplasia in the bone marrow

supported the latter mechanism. Hirokawa *et al.* identified a total of 22 patients with lymphoma-associated PRCA worldwide in the literature between 1978 and 2007, 7 of whom had a positive Coombs' test.⁷ Choi *et al.* described an additional case of angioimmunoblastic T-cell lymphoma associated with PRCA and a positive Coombs' test, both of which were resolved with CHOP chemotherapy together with the underlying lymphoma.²⁰ These findings suggest that autoantibodies targeting the differentiation stages of both mature erythrocytes and erythroid precursors can be concurrently produced in the setting of lymphoid tumors. In the present case, rituximab appeared to have exerted its effects not only on neoplastic B-PLL cells, which strongly expressed CD20, but also on polyclonal antibody-producing B cells, leading to the resolution of anemia and long-term CR of the underlying B-PLL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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