Morphologic and Cytochemical Observations on the Overt Leukemic Phase of Therapy-related Leukemia

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comparison of morphologic and cytochemical features of the leukemic cells of patients with overt acute nonlymphocytic leukemia following therapy for primary malignancies (2°-ANLL) to those of patients with ANLL de *novo* revealed several differences. No Auer rods were seen in cells of patients with 2°-ANLL, but they were seen in 54 (42%) of 129 cases of ANLL de *novo*. Only one **(4.7%)** of 21 patients with 2"-ANLL was classified as M-4 by FAB criteria, compared to **46** (36%) of 129 patients with ANLL de *novo* classified as M-4. Only one (10%) of 10 patients with 2"-ANLL had more than 10% peroxidase-positive leukemic cells, whereas 19 (95%) of 20 of a control group of patients with ANLL de *novo* of similar FAB subtypes had more than 10% positive cells. Two of 11 (18%) 2°-ANLL patients had 10% or more naphthol-AS-D chloroacetate esterase-positive cells, in contrast to nine (47%) of 19 patients with ANLL *de novo*. Nonspecific esterase activity was present in 10% or more of cells in only one (10%) of **10** patients with 2"-ANLL studied, as compared to seven (35%) of 20 of the control group. Previous reports indicate that patients with 2°-ANLL have cytogenetic abnormalities and therapeutic responses that differ from those in ANLL de *novo*; our findings indicate that there are morphologic and cytochemical differences as well. (Key words: 2°-ANLL; Therapy-related leukemia; Cytochemistry) Am J Clin Pathol 1983; **79:** 525-530

THERAPY-RELATED or secondary acute nonlymphocytic leukemia (2°-ANLL) and dysmyelopoietic syndromes (DMPS) are being recognized with increasing frequency as leukemic processes with characteristic morphologic, clinical, and cytogenetic features.[1,3,8,10,12,18,20,23,25,26,29-31] Frequently, in patients with 2°-ANLL, a preleukemic phase with hyperplasia and dysplasia in the myeloid, megakaryocytic, and erythroid series evolves into overt leukemia that is refractory to treatment. Most patients with leukemia following cytotoxic therapy for malignant or nonmalignant disease have nonrandom chromosome abnormalities, usually consisting of loss of **part** or all of chromosome number 5 or number 7 or both.[1,12,18,24-26]

In our morphologic and cytochemical analysis of 26 patients who developed 2"-ANLL or DMPS following treatment for malignant disorders, we noted considerable differences from a group of 129 patients with ANLL *de novo*. The differences seen during the overt leukemic phase form the basis of this report.

Materials and Methods

At the University of Chicago Hospitals and Clinics, 150 cases of ANLL have been studied from 1970 through July 1981. Of these, 129 had ANLL *de novo*, and the other 21 cases were considered to represent overt 2°-ANLL, *i.e.*, leukemias that occurred following chemotherapy or radiotherapy or both, for malignant diseases. Five additional patients who developed **a** dysmyelopoietic syndrome following therapy for a malignant disease, but who did not develop overt acute leukemia, are not included in this series.

All available peripheral blood smears, bone marrow aspirates, and bone marrow biopsy specimens for the entire group of ANLL patients were reviewed, and 500 cell count differentials were performed on the bone marrow aspirates. Each case of acute leukemia was classified according to the FAB criteria;[4,5,15] only those cases in which the numbers of blasts in the marrow exceeded 30% of the nucleated marrow cells are included in this report.

Since 1975, cytochemical reactions, including peroxidase, naphthol AS-D chloroacetate esterase, α-naphthyl butyrate, and/or α-naphthyl acetate with or without NaF, have been applied to 83 of the 129 cases of ANLL *de novo*. Standard cytochemical procedures were used.[17,19,32] In the group of 21 patients with 2"-ANLL, 13 patients had cytochemical studies recent enough to be analyzable for the purposes of this report, and they were compared with a control group of 20 patients with ANLL *de novo* who were in similar FAB subgroups and who had cytochemical procedures performed within the same period.

At least 100 bone marrow cells were analyzed for each cytochemical reaction. The number of immature cells

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demonstrating positive reactions was counted, and the intensity of the reaction was quantitated on a scale of 1+ to 4+ positivity. A cell was scored as 1+ for peroxidase if a definite, single peroxidase-positive granule was present in the cytoplasm; 2+ if more than one granule was present, but less than one-fourth of the cytoplasm was occupied by positive granules; 3+ if one-fourth to one-half of the cytoplasm. showed reaction product; and 4+ if the reaction was intense and positive granules were present throughout the cytoplasm.

Cells that were considered to be immature leukemic cells were tallied; the degree of chromatin condensation was used as the primary criterion of immaturity. Nevertheless, the tally probably reflects not only blasts but also some promyelocytes, since these two cell types cannot always be distinguished in the cytochemical procedures. Reactivity in neutrophils was tallied separately, but in some cases, neutrophils were so scarce that a meaningful interpretation was not possible. A similar scoring procedure was used for the naphthol AS-D chloroacetate esterase (NASDCA) and acid phosphatase (AcP) procedures. Although both a-naphthyl butyrate and a-naphthyl acetate esterases with and without NaF reactions were available for most patients, more 2°-ANLL patients had been studied with the butyrate substrate; we chose, therefore, that reaction to assess the nonspecific esterase (NSE). (In our laboratory, α-naphthyl butyrate and a-naphthyl acetate give similar results.) Primitive cells demonstrating NSE activity were scored according to the intensity of the reaction that was noted diffusely in the cells. The number of immature cells showing NSE activity, as well as the total number of positive cells, was scored. No case was considered to represent acute myelomonocytic leukemia (M-4) unless there were more than 20% monocytoid cells in the blood and/or bone marrow, and unless this finding was confirmed by a similar number of nonspecific esterase-positive cells.

The periodic-acid-Schiff reaction was scored in both the immature leukemic cells and the erythroid precursors.

Transmission electron microscopy (TEM) was used for the study of cases in which the nonlymphocytic nature of the leukemic process was in doubt because of poor cytochemical reactivity of the neoplastic cells. Ultrastructural cytochemical studies were not performed routinely.

Results

Clinical

The clinical, morphologic, and cytogenetic findings in all except four of the 21 patients with 2°-ANLL have been detailed elsewhere.[25,26,30] Briefly, 14 had been treated previously for Hodgkin's disease (HD), three for small cleaved follicular center cell lymphoma (PDL), two for multiple myeloma (MM), and two for carcinoma. Sixteen patients had received both radiotherapy and chemotherapy; three, radiotherapy alone; and two, chemotherapy alone. The mean age at the onset of leukemia was 43 years. A preleukemic period was documented in 13 (62%) of the patients. Examination of the peripheral blood and bone marrow smears and biopsy specimens of patients during the preleukemic period demonstrated many features in common with a myelodysplastic syndrome, very similar to the features of refractory anemia with excess blasts (RAEB), or RAEB in transformation, as described in the FAB classification.[4,5,15] The numbers of immature cells and the degree of dysplasia increased as each patient evolved toward an overt leukemic phase, defined as more than 30% blasts in the bone marrow.

In the 129 patients with ANLL *de novo,* the mean age at the time of diagnosis was 48 years. A preleukemic phase was documented in very few patients, if any.

Classification of Leukemia

Of the 129 patients with ANLL *de novo,* 28 (22%) were classified as M-1 by the FAB criteria, 34 (26%) as M-2, and 10 (8%) as M-3. Acute myelomonocytic leukemia (M-4) was diagnosed in 46 (36%) of these patients, whereas M-5 was found in five (4%). Erythroleukemia (M-6) was present in five (4%) patients (Table 1). One case was unclassifiable because of extensive marrow necrosis. The mean blast count in these patients was 54%, with a range of 30–90%. The mean marrow cellularity was 88%, with a range of 30–100%. as evaluated from marrow biopsy specimens.

Classification of the cases of 2°-ANLL according to the FAB criteria was difficult in approximately 70% of the cases. These cases showed atypical features, such as clear evidence of trilineage involvement (70%), many micromegakaryocytes and/or evidence of megakaryocytic proliferation (70%), and moderate to markedly increased marrow reticulin (47%). Additionally, the appearance of very primitive blasts in a background of dysplastic myeloid and erythroid elements often gave the impression that the leukemia might later be characterized by primitive, poorly differentiated cells if it were allowed to run its course. The mean marrow cellularity was 87%, as judged from bone marrow biopsy, with a range of 50–100%. The mean blast count at the time of overt leukemia was 53%, with a range of 30–95%, for those patients in whom countable aspirates were available. It frequently was difficult to decide when the disease had evolved from a myelodysplastic state

into overt leukemia. Although the number of blasts was used as the basis for this decision, the presence of marrow fibrosis in some cases did not permit accurate quantitation of the blasts. Of the 21 patients who developed ANLL secondary to therapy, four (19%) were classified as M-1; 11 (52%) were M-2, one (5%) was **M-3;** and four (19%) were **M-6.** Only one **(5%)** patient was classified as M-4, and no cases of acute monocytic leukemia (M-5) were detected (Table 1).

Auer rods were present in **54 (42%)** of the 219 patients with ANLL *de **novo*** but were not seen in any of our patients with 2°-ANLL. In the single case of 2°-ANLL classified as **M-3,** more than 50% of the cells had heavy azurophilic granulation, and some had the bi-lobed, reniform nuclei typical of promyelocytic leukemia; however, Auer rods were not detected on the marrow aspirate smears.

Table 1. Distribution of Patients in the Present Study According to the **FAB** Classification

Type	ANLL *de novo*		2°-ANLL	
	Number of Patients	%	Number of Patients	%
M-1	28	22	4	19
M-2	34	26	11	52
M-3	10	8	1	5
M-4	46	36	1	5
M-5	5	4	—	—
M-6	5	4	4	19
Unclassified	1			
Total	129		21	
Auer rods	54	42	0	0

Cytochemical Findings

Only the findings in 13 2°-ANLL patients are included in this report, since the procedures on the other eight patients either had been performed before this study began and were considered unreliable for the scoring method used because the reaction product had faded, or had been performed on touch preparations from fibrotic marrows, and there were too few cells to score reliably. Of the **13** patients with 2°-ANLL, four had been classified as M-1, seven as **M-2.** one as **M-4,** and one as M-6. Twenty patients with similar classifications of ANLL *de **novo*** were studied by cytochemistry during the same period, and these served as a control group. Of the 20 patients with ANLL *de **novo,*** three were M-1, nine were **M-2,** six were **M-4,** and two were **M-6.**

In the group of 2°-ANLL patients. only one (10%) of 10 patients studied for peroxidase activity had more than 10% peroxidase-positive immature leukemic cells **(64%** of the cells were positive), and seven of the 10 patients had only **3–9%** peroxidase-positive cells (Table 2). The remaining two patients had only 2% peroxidase-positive immature cells, and the diagnosis of **M-1** was confirmed by TEM studies. In contrast, 19 **(95%)** of the 20 patients in the control group had more than 10% peroxidase-positive immature leukemic cells (Fig. 1). No significant difference between the two groups was found, however, in the percentages of peroxidase-positive or peroxidase-negative neutrophils.

The intensity of the peroxidase reaction also differed between the 2°-ANLL patients and the control group. Only one (10%) of **10** of the patients with 2°-ANLL had any immature cells scored as **3+.** Although eight of the 10 had some cells that were scored as high as 2+, more than 50% of the peroxidase-positive cells in five patients were only 1+. In contrast, 14 of the 20 patients with ANLL *de **novo*** had peroxidase-positive immature cells that were scored as **3+** or higher, and in 11 cases, more than 50% of the immature cells were scored as 2+ or higher.

The naphthol AS-D chloroacetate esterase reaction was positive in more than 10% of the immature leukemic cells in two (18%) of 11 2°-ANLL patients (Table **2).** In one of these patients classified as **M-2,** 26% of the cells were positive with the NASDCA reaction, and **64%** were positive with the peroxidase reaction. In the second patient, there were 28% NASDCA-positive cells, but only 9% peroxidase-positive cells. In seven of the remaining 2°-ANLL patients, some positive cells were present, but the NASDCA reactions were very weak, and all positive cells in each of these patients were scored as only 1+.

In the control group, nine **(47%)** of 19 patients studied with NASDCA had more than 10% positive immature cells, and 16 (84%) of these had at least some cells that were scored higher than **2+.**

A difference in the number of peroxidase-positive cells and NASDCA-positive cells was noted in both groups of patients. One patient with 2°-ANLL and two patients in the control group had more positive cells with NASDCA than with peroxidase; in general, however, in both groups of patients, far fewer cells were positive with the NASDCA than were positive with the peroxidase

Table 2. Results of Cytochemical Studies

	2°-ANLL	Control Group with ANLL *de novo*
>10% Peroxidase-positive cells	1/10 (10%)	19/20 (95%)
>10% Chloroacetate-esterase-positive cells	2/11 (18%)	9/19 (47%)
>20% Nonspecific esterase-positive cells	1/13 (8%)	*6/20 (3046)*
>10% Acid-phosphatase-positive cells	8/8 (100%)	20/20 (100%)

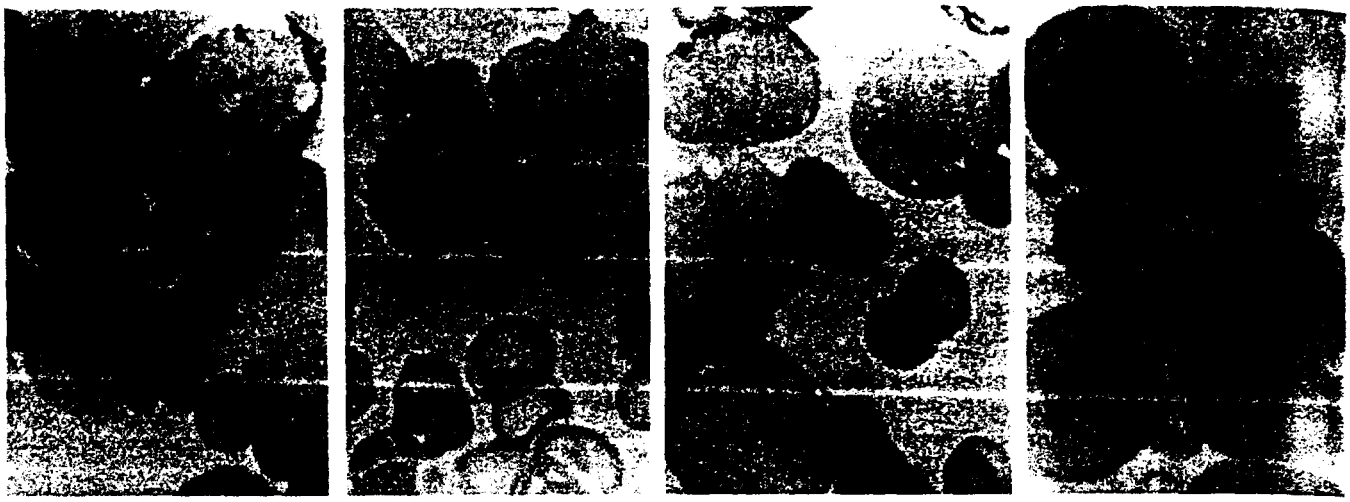

FIG. 1 (*left*). **The cytochemical differences that were typical between 2°-ANLL and ANLL *de novo* are illustrated in this photomicrograph. Peroxidase-positive blasts are readily identified in the case of ANLL *de novo* (Fig. 1*A, far left*), whereas the blasts from a patient with 2°-ANLL (Fig. 1*B, left, center*) show no reactivity. (A neutrophil serves as a positive control.) Peroxidase (×1,000).**

FIG. 2 *(righf).* **In this study, it was generally noted that there was a greater sensitivity of the peroxidase reaction than the NASDCA reaction in leukemic cells. This figure compares the peroxidase reaction (Fig. 2*A, righf. cenfer*) with the NASDCA reaction (Fig. 2 *E. far righf*) from the same patient with M-I ANLL *de novo*. Most of the immature cells show some peroxidase activity. but only a more mature granulocytic cell shows NASDCA activity. Peroxidase and NASDCA (×1,000).**

reaction. Fifty-four per cent of the patients in both groups had percentages of peroxidase-positive cells more than twice the percentage of NASDCA-positive cells (Fig. 2).

Only one 2"-ANLL patient had more than 10% positive cells with NSE (Table 2); **46%** of the total nucleated marrow cells were found to be positive, and the morphologic classification was **M-4.** In the control group, all six of the patients classified as **M-4** had more than 20% NSE-positive cells, and none of the remaining patients had more than 10% positive cells.

Diffusely distributed acid phosphatase activity was present in all eight patients with 2"-ANLL who were studied for that reaction, and in all 20 patients in the control group. In five (62%) of the cases of 2°-ANLL, more than 50% AcP-positive cells were tallied, whereas 16 (80%) of the patients in the control group had more than 50% AcP-positive cells. In general, the intensity of the reaction also was stronger in the control group.

In 60% of patients with 2"-ANLL, **2–8%** of cells interpreted as nonerythroid blasts were shown to have PAS positivity in the form of fine granules; similar activity was present in 7**1%** of the control group. However, PAS activity in from 2–8% of erythroid cells was seen in 50% of the patients with therapy-related leukemia, but only 29% of the patients with ANLL ***de novo*** showed similar reactivity.

Discussion

The myelodysplastic and leukemic process following chemotherapy and/or radiotherapy has been characterized as a disorder involving the hematopoietic stem cells. with ensuing abnormalities in the myeloid, monocytic, erythroid, and megakaryocytic series.[10,12,20,23,30] Recent evidence indicates that, in some cases, lymphoid cells may participate in the leukemic process as well.[24] More than half of the patients who develop 2"-ANLL have a preleukemic phase of variable duration that is characterized by cytopenia, macrocytic anemia, and severe dysplasia in the cells of the peripheral blood and bone marrow. Morphologically, these changes resemble RAEB, or RAEB in transformation.[4,5,15] Chromosome abnormalities, most often consisting of hypoploidy, with abnormalities frequently involving chromosome number 5 or number 7 or both, are present almost invariably.[23,25,26] During the preleukemic phase, there is often a gradual increase in the number of immature cells until an overt leukemia emerges.

Comparison of the morphologic and cytochemical features of the overt leukemic phase of 2"-ANLL with those of ANLL ***de novo*** revealed several differences in the patients studied at our institution. Patients with 2°-ANLL often were more difficult to classify by FAB criteria, and 70% showed evidence of considerable trilineage involvement. There were fewer patients with therapy-related disease, however, who showed significant participation of monocytes in the leukemic process. and Auer rods appeared to be uncommon. In addition, the numbers of leukemic cells that demonstrated cytochemical reactivity for myeloid differentiation enzymes. such as myeloperoxidase and naphthol AS-D chloroacetate esterase, were fewer in 2"-ANLL than in ANLL ***de novo.***

In several recent reports, the number of patients with

2°-ANLL who had either M-4 or M-5 leukemia appeared to be fewer than expected,[12,20,22,23,27] although Griinwald and Rosner state that M-4 is the most common type of 2"-ANLL following therapy for Hodgkins disease.[16] In a detailed morphologic report by Foucar and associates,[12] two of 15 patients with 2"-ANLL were described as having a minor monocytic component in their leukemia. McKenna and co-workers[20] reported on 13 2°-ANLL patients who were studied ultrastructurally. In only two cases promonocytes predominated; abnormalities in the monocytic series were noted in additional patients, however, providing evidence for a panmyelocytic disease process. In a report by Pedersen-Bjergaard and colleagues,[23] two of 19 patients with overt 2°-ANLL were classified as M-4, according to the FAB criteria, and none were classified as M-5. Mitelman and associates[21] found that in acute nonlymphocytic leukemia related to occupational exposure to toxic agents, monocytic varieties of ANLL were found in only 17.4% of exposed patients, compared to 42.4% of nonexposed patients. Likewise, the incidence of karyotypic abnormalities, including missing chromosomes 5 and 7, was higher in the exposed than in the nonexposed group in that study and thus somewhat similar to the incidence in patients with 2°-ANLL. An analysis of 60 cases of overt 2"-ANLL from the Fourth International Workshop on Chromosomes in Leukemia showed 10 (16%) cases of M-4, whereas 179, or 21%, of 860 ANLL *de novo* cases were classified as M-4,[13] although this difference is not statistically significant.

However, a review of 147 reported cases of 2°-ANLL, in which sufficient data are available for determination of the leukemic subtype, showed that 28% of the cases were classified as myelomonocytic or monocytic types. Most of these cases were not classified according to the FAB criteria, however, and very few were studied cytochemically. One notable exception is the study of Kapadia and colleagues[18] in which nine (45%) of 20 cases were classified as M-4. Nevertheless, in that report, only "occasional" leukemic cells were described as positive with the nonspecific esterase reaction in patients Classified as M-4.

It would appear, therefore, that careful analysis of a larger group of patients is needed before we can determine whether our observation of a decreased incidence of monocytic varieties of leukemia in 2°-ANLL, as compared with ANLL ***de novo,*** is valid and. whether this observation bears any relationship to cytogenetic findings or is of any significant clinical value. The incidence of myelomonocytic leukemia and monocytic leukemia in our patients with ANLL ***de novo*** is similar or slightly greater than that reported by other groups,[7,11,14] indicating that we do not underdiagnose this type of leukemia.

That the cytogenetic changes observed in leukemia may have some bearing on the morphologic expression of the process recently has been highlighted by Rowley and associates[27] in a report that relates the morphologic type of ANLL to the karyotypic changes in 503 patients with ANLL ***de novo.*** In that study, patients who were lacking a chromosome 5 or 7 or both—the changes most frequently observed in 2"-ANLL—were noted to have the M-1, M-2, or M-6 type of leukemia, whereas patients with M-4 or M-5 rarely showed this karyotypic pattern.

None of our patients with treatment-related leukemia had Auer rods, although they were found in 42% of our patients with ANLL ***de novo,*** an incidence similar to that reported in the literature for other series of patients with ANLL ***de novo.***[2,14,21,28] It is difficult to ascertain how many cases of 2°-ANLL reported in the literature were observed to have Auer rods; however, we found 160 cases that were described adequately, and Auer rods were mentioned in only 17 (11%).

Of our larger group of 83 patients with ANLL ***de novo*** studied by cytochemistry, 7% have shown fewer than 10% peroxidase-positive leukemic cells, and some have required additional studies, such as electron microscopy, for confirmation of the nonlymphoid origin of their leukemia; these cases have been in either the M-1 or M-5 category. The finding in our patients with 2"-ANLL that the numbers of cells that were reactive in the peroxidase and naphthol AS-D chloroacetate esterase reactions were smaller than in the control group indicates that *(1)* the leukemic cells are of more primitive granulocytic origin, ***(2)*** that they are of diverse origin with light microscopic features indistinguishable from those of myeloblasts, or ***(3)*** that their enzyme content is abnormal.

The finding, in 2"-ANLL, of decreased numbers of cells positive for the cytochemical markers usually present in myeloid leukemias is not unexpected, since the secondary bone marrow neoplasia represents a panmyelosis, with participation of cells of the erythroid and megakaryocytic as well as granulocytic series. Evolution of such a process may well result in a leukemia of very primitive cells derived from precursor cells of diverse origin, as well as from stem cells. Sultan[29] has described four patients with treatment-related leukemia who had significant numbers of megakaryoblasts in their marrow and peripheral blood, which could be identified only by application of the ultrastructural platelet peroxidase technic described by Breton-Gorius.[6] Additionally, McKenna[20] described two patients with immature cells that proved in ultrastructural studies to be basophils, but which could not be identified as such by light microscopy. Thus, it appears that the immature cells in patients with overt 2"-ANLL may be very primitive and of diverse origin. Our cytochemical studies support this view. Ultrastructural cytochemistry, which was not used routinely in our cases, may lead to a better definition of the cell types involved.

In this study, the peroxidase reaction appeared to be more sensitive than the naphthol AS D chloroacetate esterase reaction in detecting leukemic cells of granulocytic origin. In the control group of 20 patients with ANLL *de novo* studied at the same time as our 2°-ANLL patients, only one (5%) patient had fewer than 10% peroxidase-positive immature cells, whereas 53% had fewer than 10% NASDCA-positive cells.

The poor cytochemical reactivity of the blasts in our patients with 2°-ANLL is similar to that described in chronic myelogenous leukemia (CML), in which, even in a blast crisis involving morphologically granulocytic cells, only a small percentage of the blasts may show peroxidase activity.[9] There are other similarities between the blast phase of CML and that of 2°-ANLL, including a preceding myeloproliferative phase, rarity of Auer rods; the presence of nonrandom chromosome changes, and resistance to therapy. These two diseases probably reflect stem cell abnormalities with similar terminal manifestations.

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