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Adult T-Cell Leukemia/Lymphoma in Northeastern Brazil: A Clinical, Histopathologic, and Molecular Study

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Summary: The state of Bahia in the northeastern coast of Brazil is a region in which HTLV-I infection is endemic. This study investigated the characteristics of 28 HTLV-I–associated lymphomas/leukemias in this region. HTLV-I–infection diagnosis was based on serologic study, Southern blot analysis, and polymerase chain reaction (PCR) in neoplastic tissue. The main clinical differences between these lymphomas and adult T-cell leukemia (ATL) cases from other endemic areas were as follows. The mean age was 47 years; 20% of the cases occurred in young adults; and a predominance was found among male subjects (2:1), blacks, and of those of mixed race (96%). Histologically, 20 cases were T-cell pleomorphic leukemia/lymphoma, 5 were *Mycosis fungoides*–like cutaneous lymphoma, and 3 were $CD30^+$ large-cell anaplastic lymphoma. Immunohistochemistry demonstrated 4 cases of $CD8^+$ lymphoma. Proviral genomic sequences were demonstrated by PCR in 9 lymph node biopsy specimens and in 3 skin biopsy specimens. Southern blot was performed and was positive in 8 cases.
Key Words: HTLV-I–positive lymphoma—Adult T-cell leukemia/lymphoma—Pleomorphic lymphoma.

Adult T-cell leukemia/lymphoma (ATL) was first recognized as an aggressive lymphoma with characteristic cell morphology by Uchiyama et al. in 1977 in Japan (1). A few years later in 1981, Poiesz et al (2) identified the first human retrovirus, HTLV-I, isolated from a patient with cutaneous T-cell lymphoma (CTCL). Shortly afterward this same C-type retrovirus was isolated from an ATL cell line in Japan (3) and, subsequently, several seroepidemiologic studies demonstrated the association between HTLV-I and ATL (4,5). Further evidence of the important role of HTLV-I in ATL was achieved by demonstration of monoclonal integration of the HTLV-I provirus genome in tumor cells of ATL cases (6).

HTLV-I is endemic in many regions including Southwest Japan (7), West and Central Africa (8), the Caribbean basin (9), the southeastern United States (10) and South America (11). In Brazil, the highest seroprevalence rate in blood donors and healthy subjects (1.3%–1.8%) was observed in the state of Bahia (12,13), situated on the northeastern coast and with a large population of African descent. In addition, in a recent publication from Rio de Janeiro (Brazil), 26.5% of the patients with T-cell malignancies were serologically positive for HTLV-I and represented ATL cases (14). However, little is known about the importance of this

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infection in the pathogenesis of lymphoma in endemic regions like the northeastern coast and other areas of Brazil, although isolated reports of ATL cases have been made (15).

In the present study, clinical, histopathologic, and immunohistochemical features of 28 cases of HTLV-I–positive lymphomas from Bahia-Brazil are presented.

MATERIALS AND METHODS

From 1991 to 1995, all cases of T-cell peripheral lymphoma, referred to the Department of Pathology of the University Hospital, which showed histologic/clinical characteristics suggesting ATL or cutaneous lesions were investigated for HTLV-I infection. In all, 28 HTLV-I associated lymphomas were diagnosed and included in this study. All patients were born in the state of Bahia.

Clinical data were collected from the records and included physical examination, hemograms, chest radiography, abdominal ultrasonography, calcium and lactodehydrogenase blood levels, bone marrow aspiration, and biopsy. Duration of disease was defined as the interval between the first clinical manifestations and histologic diagnosis. Survival was characterized as the interval between histologic diagnosis and death or last follow-up (November 1996). Clinical subtypes were classified according to Shimoyama's criteria (16). Cutaneous histologic features of 17 of the HTLV-I–positive cases of the present series have been compared with equal number of HTLV-I–negative cutaneous lymphomas in a previous paper (17). One case (case #27) has been previously described in the series reported by Pombo de Oliveira and associates (14).

Histopathology and Immunohistochemistry

All cases had surgical biopsies of skin lesions, lymph nodes, or both. An autopsy was performed in 1 patient and all organs were examined (case #26). Histologic sections were stained with hematoxylin-eosin, Gomori's silver impregnation, and Lennert's Giemsa. Following the revised European-American classification of lymphoid neoplasms (REAL) classification, all HTLV-I–associated lymphomas included in this study should be classified histologically as ATL (18). However, the HTLV-I–positive lymphomas that showed histologic characteristics of other recognized entities of the REAL classification were subclassified accordingly (18). Lymphomas with typical ATL morphology were designated as pleomorphic lymphomas (PL).

Immunocytochemical study of the neoplastic cells was performed on formalin-fixed, paraffin-embedded sections using a panel of antibodies and a standard streptavidin-biotin-peroxidase technique (19). The following immunocytochemical markers were employed: T-cell markers CD45R0, CD3, CD8 and OPD4; B-cell markers CD20, CD45R (4KB5); Reed-Sternberg cell and activated B-cell and T-cell marker CD30; antihuman epithelial membrane antigen antibody (EMA). More than 50% of the neoplastic cells had to be stained to be classified as positive to a T-cell marker.

Serology

Sera were collected and the following serologic assays were performed: antibodies to HTLV-I/II were investigated by diagnostic enzyme-linked immunosorbent assay (ELISA) (HTLV-I rp21e–enhanced ELISA, Cambridge Biotech, Worcester, MA, U.S.A.). Repeat reactive samples were confirmed with Western blot capable of discriminating HTLV-I and II (HTLV Blot 2.4, Genelab, Singapore). Results were interpreted according to the manufacturer's instructions. Briefly, reactivity to *gag* (p19 with or without p24) and two *env* (GD 21 and rgp46-I) bands was considered as HTLV-I positivity. Cases that showed positivity for HTLV-specific bands but did not meet criteria for HTLV-I or II or HTLV positivity were considered as indeterminate.

All HTLV-I–positive patients were serologically tested for HIV by ELISA (Dupont, Wilmington, DE, U.S.A. and Innogenetics, Belgium).

Polymerase Chain Reaction in Paraffin Block Tissue

High-molecular-weight DNA was extracted from paraffin-embedded tissue according to the procedure described by Wright and Manos (20) or using QIAamp tissue kit (Qiagen GmbH, Hilden, Germany) according to the instructions from the manufacturer. Two sets of primers were employed: one for the *pX* region and the other for the *pol* gene:

pX up: 5′- CGGATACCCAGTCTACGTG-3′
pX low: 5′- ATAAGGAACTGTAGAGCTGAGCCGATAA35
Pol up: 5′- CGGATACCCAGTCTACGTG-3′
Pol low: 5′- ATAAGGAACTGTAGAGCTGAGCCGATAA35

PCR amplification of DNA was performed following the procedures described by Anagnastopoulos and colleagues (21). Briefly, 100 ng of each sample DNA was amplified in a buffer mixture (100 μl) containing 10 mMol/Tris-HCl (pH 8.3), 50 mMol/L KCl, 1.5 mMol/L $MgCl_2$, 200 μMol/L (micromolar) each deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), 0.2 Mol/L of the selected primer, and 2.5 U of Taq DNA polymerase (AmpliTaq-Elmer Cetus, Norwalk, CT, U.S.A.). HUT-102 cell line was used as a positive control. After an initial denaturation at 96°C for 4 minutes, 40 cycles of the PCR were carried out. Each of these cycles consisted of denaturation at 94°C for 30 seconds and annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The extension in the final cycle lasted 10 minutes. The final amplification products were examined on a 5% polyacrylamide agarose gel stained with ethidium bromide. A tube without DNA sample and HTLV-I negative cell lines Ag876 and B-95 served as negative controls.

Southern Blot Analysis

Southern blot assay to detect clonal integration of HTLV-I proviral DNA in peripheral blood was made in 8 cases (cases #2, #3, #4, #5, #14, #15, #16, and #17). High-molecular-weight DNA was extracted from peripheral blood mononuclear cells (PBMCs). DNA samples were digested with the restriction enzymes *Bam*HI, *Hind*III and *Eco*RI (New England Biolabs, Beverly, MA, U.S.A.) for 16 hours at 37°C and then fractioned according to size on 0.8% agarose gel, transferred to nitrocellulose and hybridized with P-labeled full-length HTLV-I complementary DNA (22).

RESULTS

In the result, 27 lymphoma cases were seropositive by ELISA and Western blot. In 1 case, PCR demonstrated

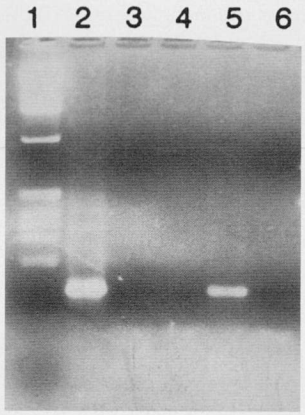

FIG. 1. Polymerase chain reaction analysis of cases #7, 8, and of case #22, which was the only HTLV-I–seronegative case. Amplification products using HTLV-I specific primer from the px region. Lane 1, standard; lane 2, positive control (HUT102 infected cell line, fragment length 160bp); lane 3, case 7, negative; lane 4, case 8, negative; lane 5, case 22, positive px gene; lane 6, negative control (H_2O).

the presence of HTLV-I genomic sequences in neoplastic tissue (Fig. 1) although ELISA and Western blot serologic results were negative (case #22). Only 1 case was seropositive for HIV (case #18).

Polymerase Chain Reaction in Neoplastic Tissue

HTLV-I genomic sequences were demonstrated in 12 cases: 9 in lymph node tissue (Fig. 2) and 3 in skin biopsy specimens (Table 1). In all other skin biopsy specimens, insufficient DNA was extracted and amplification was not possible. Negative results were obtained in 2 of the lymph node biopsy specimens, possibly as a result of inadequacy of the tissues.

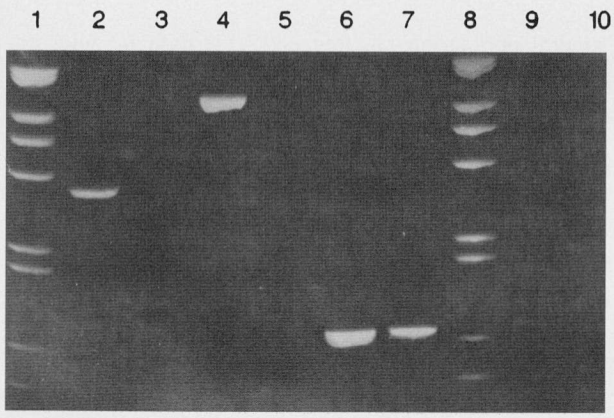

FIG. 2. Polymerase chain reaction analysis of case 23. Amplification products using HTLV-I specific primers from the *gag*, *pol*, and *px* regions. Lane 1, standard; lane 2, positive control, gag gene (HUT102 infected cell line, fragment length 270 base pairs [bp]); lane 3, case 23, negative for *gag*; lane 4, positive control, *pol* gene (amplified product length 391 bp); lane 5, case 23 negative for *pol*; lane 6, positive-control *px* gene (amplified product length 160 bp); lane 7, case 23, positive, *px* gene; lane 8, standard; lane 9 negative control (H_2O); lane 10, negative control (B-95 cell line).

Southern Blot Analysis

Clonal integration of HTLV-I was found in the PBMCs of all 8 patients studied (Table 1).

Clinical and Laboratory Features of HTLV-I–Positive Lymphomas

As stated previously, all patients had been born in the state of Bahia. As the study involves race, 13 were black, 14 mixed race, and 1 white. The male:female ratio was 2:1. The median age was 47 years, with a range of 13 to 80 years. Seventeen patients died and 11 are alive with a median follow-up period of 21 months. Overall median survival was 1.3 years. Male:female ratio was 8 in the leukemic forms of ATL, which include acute and chronic subtypes and 1.4 in nonleukemic forms, comprising the smoldering and lymphoma types. Clinical subtype, histologic diagnosis, and biopsy site are shown in Table 1. Demographic characteristics are summarized in Table 2. Laboratory data according to clinical subtype are shown in Table 3. Other characteristics are described as follows:

Histology

Twenty cases (71.4%) were T-cell pleomorphic lymphomas commonly described in association with HTLV-I, composed of a mixture of small, medium, and large cells with nuclear pleomorphism as described in ATL (18). Polymorphism, Reed-Sternberg–like cells and giant cells with "jellyfish" or cerebriform nucleus were observed more frequently in large-cell or medium-and-large-cell types. Small- and medium-cell PL showed little polymorphism and were constituted of cells of equal size admixed with a few large cells, similar to immunoblasts or Hodgkin's cells. In Case #20, extensive areas of neoplastic tissue were represented by CD30-positive large round cells. In skin biopsy specimens, dermal infiltration by neoplastic pleomorphic cells were observed. In 75%, epidermotropism was present and in 58% Pautrier's abscesses were also present

In 3 cases (10.7%), the neoplastic tissue was seen as cohesive sheets of large round cells with round vesicular nuclei and a solitary, eosinophilic, or amphophilic nucleolus, frequent multinucleated cells, and many Reed-Sternberg–like cells. More than 90% of the neoplastic cells were $CD30^+$-positive in these cases. They

TABLE 1. *Clinical subtype, histologic diagnosis, polymerase chain reaction (PCR) results, and biopsy site of 28 HTLV-I–positive lymphoma cases*

Case	Age	Gender	Race	Clinical subtype	Histopathology	Biopsy site PCR and Southern blot positivity
1[c]	20	M	Black	Lymphoma	Pleomorphic L	Skin, node
2[c]	44	M	Black	Chronic	Pleomorphic M	Skin[a]
3[c]	42	M	Black	Acute	Pleomorphic M/L	Skin, node[a]
4	30	M	Black	Smouldering	Pleomorphic M	Skin[a]
5	13	M	Mixed race	Smouldering	Pleomorphic M	Skin[a]
6[c]	58	M	Black	Smouldering	Pleomorphic M/L	Skin
7[c]	51	M	Black	Acute	Pleomorphic M/L	Skin, node
8[c]	54	M	Black	Acute	Pleomorphic M/L	Skin, node
9	56	W	Mixed race	Smouldering	Pleomorphic M	Skin
10	40	W	Black	Smouldering	Pleomorphic M	Skin
11[c]	52	M	Mixed race	Acute	Pleomorphic M	Skin[b]
12	62	W	Mixed race	Smouldering	Pleomorphic L	Skin
13[c]	49	M	Mixed race	Acute	MF-like	Skin
14	70	M	Mixed race	Smouldering	MF-like	Skin[a,b] (14)
15	28	M	Mixed race	Chronic	MF-like	Skin, node[a]
16	67	M	Black	Smouldering	MF-like	Skin[a]
17	42	W	Mixed race	Smouldering	MF-like	Skin[a]
18[c]	44	M	Black	Lymphoma	$CD30^+$ LCAL	Skin[b]
19[c]	18	W	Black	Lymphoma	$CD30^+$ LCAL	Node[b]
20[c]	40	M	Mixed race	Lymphoma	Pleomorphic L	Node[b]
21[c]	80	W	Mixed race	Lymphoma	$CD30^+$ LCAL	Node[b]
22[c]	59	M	Black	Lymphoma	Pleomorphic M/L	Node[b]
23[c]	67	M	Black	Lymphoma	Pleomorphic L	Node[b]
24[c]	66	W	Mixed race	Lymphoma	Pleomorphic L	Node[b]
25[c]	48	M	Mixed race	Lymphoma	Pleomorphic S/M	Node[b]
26[c]	22	W	Mixed race	Acute	Pleomorphic M/L	Node[b]
27	44	M	White	Lymphoma	Pleomorphic M	Node[b]
28	54	W	Mixed race	Lymphoma	Pleomorphic M/L	Subcutaneous tissue

[a] Southern blot–positive.
[b] Positive result, polymerase chain reaction.
[c] Deceased.
PCR, polymerase chain reaction; m, man; w, women; L, large cell; M, medium cell; MF, mycosis fungoides; LCAL, large cell anaplastic lymphoma; S, small cell; node, lymph node involvement.

were classified as $CD30^+$ large-cell anaplastic lymphoma.

In 5 cases (17.8%), skin biopsy specimens showed histologic features similar to *Mycosis fungoides*. Small cerebriform cells and infrequent atypical large cells were observed in the dermis. Two of these cases had atypical small cerebriform cells in peripheral blood. Epidermotropism was present in 4 and Pautrier's abscesses in 3 of these cases.

Immunohistochemistry

Immunohistochemical results are summarized in Table 4. All cases were negative for CD15, epithelial membrane antigen (EMA), and cytokeratines.

DISCUSSION

Northeastern Brazil is considered a region endemic for HTLV-I infection, although seroprevalence rates in the general population are lower (12,13) than in other endemic regions such as Jamaica (23) and southwestern Japan (24). The present study confirms the presence of ATL in the state of Bahia and demonstrates that HTLV-I–associated lymphomas/leukemias have some distinct characteristics in this geographic area. The median age was a decade younger than in ATL cases in Japan (7) and similar to Caribbean cases described (25). Indeed, ~20% of the cases occurred in young adults ≤30 years of age, and 1 patient was a 13-year-old child, whereas in Japan, 90% of ATL patients are >40 years of age (7). A male predominance (2:1) was evident in this series, whereas in Japan (16) and the Caribbean basin (25), practically no difference is found between the genders. In addition, an evident male predominance was identified in the leukemic forms of ATL, which include the acute and chronic types whereas nonleukemic disease of smoldering and lymphoma types predominated in female subjects. Overall medium survival of ATL cases was slightly higher than in Japan (7); a few cases of exceptionally prolonged

TABLE 2. *Demographic and clinical characteristics of HTLV-I–positive adult T-cell leukemia cases according to clinical subtypes*

Characteristic	Acute (n = 7)	Chronic (n = 2)	Lymphoma (n = 10)	Smouldering (n = 9)	All cases (n = 28)
Age range (y)	22–54	28–44	18–80	13–80	13–80
Medium age (y)	45	36	49	48	47
Medium survival (y)	0.4	5.8	1.2	1.3[a]	1.3
Medium duration of disease (y)	0.4	2.5	0.3	4.6	2.0
Death during follow-up	7	1	8	1	17
Ethnicity					
Black	3	1	5	4	13
Mixed race	4	1	4	5	14
White	0	1	0	0	1
Men	6	2	6	5	19
Women	1	0	4	4	9
Lymphocytes × 10^9L)	1.8–200	31–98	1.0–1.8	1.5–5.4	1.0–200

[a] Medium survival or follow-up.

disease, up to 20 years, were noted among chronic and smoldering cases.

The state of Bahia has a population of diverse ethnology including European, South American Indian, and African descent, part of the population being of mixed European and Indian background. In the present study, most patients were of African descent, thus giving support to the concept of an African origin for HTLV-I infection in Brazil and South America (24).

PCR in neoplastic tissue demonstrated the presence of HTLV-I provirus sequences in 1 case of seronegative T-cell pleomorphic lymphoma. Previous studies have described the occurrence of seronegative ATL cases in endemic areas, in which provirus sequences in neoplastic cells were demonstrated by PCR or Southern blot (26). A possible explanation for the absence of viral antibodies could be that only a fraction of the virus genome is present in neoplastic cells, most frequently *tax* and, for this reason, not all virus proteins would be produced, leading to a negative serology (26,27). Otherwise, it is also possible that genetic traits of the host may be responsible for the development of immune tolerance as it has been demonstrated experimentally in HTLV-I infection (28). Most lymph node biopsy specimens were positive for HTLV-I by PCR, but in skin biopsy specimens, the amount of tissue available was frequently insufficient. PBMCs were available for Southern blot analysis in 8 cases and all were positive for HTLV-I sequences.

As it refers to histologic features, HTLV-I–associated leukemia/lymphoma was described, at first, as a pleomorphic T-cell lymphoma (29). In the REAL classification, it was included as a separate entity, denominated ATL, and described as a T-cell neoplasm with variable morphology presenting usually as a diffuse lymphoma composed of cells with pronounced polymorphism and nuclear pleomorphism (18). In fact, most cases in this series showed the histologic features mentioned above. However, other histologic types of lymphoma with different characteristics have been described in association with HTLV-I infection, specially $CD30^+$ large cell ana-

TABLE 3. *Laboratory alterations and affected organs of 28 HTLV-I–positive lymphomas according to clinical subtype*

Involved organ/alteration	Acute (n = 7)	Lymphoma (n = 10)	Chronic (n = 2)	Smouldering (n = 9)	Total	Percentage
Lymph node	7	9	2	0	18	64.2
Skin/subcutaneous tissue	6	7	2	9	24	85.7
Hepatomegaly	7	6	2	0	15	53.8
Splenomegaly	7	6	2	0	15	53.8
Bone marrow infiltration	6	0	1	0	7	25.0
Pulmonary alterations	3	0	1	0	4	14.2
Lymphocytosis	5	0	2	0	7	25.0
Atypical lymphocytes in blood	7	1	2	6	16	57.1
Hypercalcemia	6	4	0	0	10	35.7
LDH high value	7	5	2	3	17	60.7
Central nervous system	2	0	0	0	2	7.1
Myelopathy	1	0	1	3	5	17.8

LDH, lactate dehydrogenase.

TABLE 4. *Immunophenotype of HTLV-I–associaed lymphomas*

Case	CD3	CD45R0	OPD4	CD8	CD30	CD20
1	+	+	+	—	—	—
2	+	+	+	—	—	—
3	+	–/+	+	—	—	—
4	+	+	+	—	—	—
5	+	+	+	+	—	—
6	+	+	+	—	—	—
7	+	+	—	—	—	—
8	+	+	+	—	—	—
9	+	+	+	—	—	—
10	+	+	+	+	—	—
11	+	+	+	—	—	—
12	+	+	+	—	—	—
13	+	+	+	—	—	—
14	+	+	+	+	—	—
15	+	+	+	—	–/+	—
16	+	+	+	—	—	—
17	+	+	+	—	—	—
18	+	–/+	–/+	—	+	—
19	+	—	+	—	+	—
20	+	–/+	–/+	—	–/+	—
21	+	—	—	—	+	—
22	+	+	–/+	—	—	—
23	+	+	+	+	—	—
24	+	+	+	—	—	—
25	+	—	+	—	—	—
26	+	—	+	—	—	—
27	+	+	–/+	—	—	—
28	—	–/+	+	—	—	—

—, negative; +, 60%–90% positive; –/+, <50% positive.

plastic lymphoma (LCAL) (21,30–32) and MF (33,34, 35). In this study, 3 $CD30^+$ LCAL lymphomas were identified. Furthermore, 1 case of pleomorphic lymphoma seemed to be transforming into LCAL. In all 3 cases, HTLV-I proviral sequences were demonstrated in neoplastic tissue. One LCAL case was serologically positive for both HTLV-I and HIV. In this particular case, it is not possible to exclude the possibility that HIV infection played a role in the pathogenesis, inasmuch as $CD30^+$ LCAL has been exceptionally described in association with HIV infection (36). Some 5 cases of MF-like HTLV-I–positive lymphomas were identified in the present study, 4 presenting with cutaneous lesions of long duration and absence of concurrent extracutaneous disease at the time of diagnosis. The histologic features were similar to those characteristic of MF (37). Clinical and histologic similarities between MF and cutaneous ATL and the occasional impossibility of distinguishing these two entities have been discussed by many authors (17,38–41). Moreover, the question of a possible role for HTLV-I in the pathogenesis of MF is controversial. Molecular reports have suggested that MF is associated with HTLV-I or a HTLV-I–like virus (33–35,42–46). Recent studies, however, have not confirmed these findings and the possibility of HTLV-I's being a primary etiologic agent in the pathogenesis of MF has been dismissed by many authors (47–49). Nevertheless, even if the possibility of HTLV-I's being etiologically related to MF is dismissed, our findings demonstrate that, in endemic areas, HTLV-I–associated cutaneous lymphoma may present clinical and histologic features typical of MF. For this reason, MF patients should be routinely tested for HTLV-I infection in endemic regions.

In conclusion, some clinical, epidemiologic, and histologic differences were observed between ATL cases from Bahia-Brazil and other endemic areas of HTLV-I infection. The diversity of histologic presentation of ATL demonstrated in this study emphasizes the importance of performing HTLV-I serology in endemic regions in patients with T-cell lymphomas, especially in the presence of cutaneous lesions, and also in $CD30^+$ large cell anaplastic lymphoma cases.

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