In Vitro Detection of Metastases of Small-Cell Pulmonary Carcinoma to the Bone Marrow Using Monoclonal Antibodies

M. A. Goncharskaya, N. F. Orel, N. I. Perevodchikova, and E. A. Solov'eva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, No. 4, pp. 456-458, April, 1996
Original article submitted March 16, 1995

A panel of five monoclonal antibodies to surface antigens of small-cell pulmonary carcinoma was used for immunodiagnosis of metastases to the bone marrow *in vitro* in patients with small-cell carcinoma of the lungs. Standard cytomorphological diagnosis revealed metastases to the bone marrow in 6 out of 58 patients (10.3%). Using monoclonal antibodies, positive cells were detected in 12 (20.6%) patients, the tumor nature of the positive cells being undoubted in 7 preparations (12.6%). Five out of 6 cytologically positive cases were confirmed.

Key Words: *small-cell pulmonary carcinoma; monoclonal antibodies; metastases to the bone marrow; immunodiagnosis*

Small-cell pulmonary carcinoma (SPC) is responsible for about 25% of all lung cancer cases and is one of the most malignant tumors [2], which is why the detection of all metastases is of high priority, specifically, the diagnosis of metastases to the bone marrow. This is very important for the choice of therapy and the general prognosis. Study of the markers of SPC has helped obtain a great number of monoclonal antibodies (MAb) to cell surface antigens [5,6], and their use for *in vitro* immunodiagnosis of SPC metastases to the bone marrow is one of the burgeoning trends in the diagnosis of this disease [3].

We obtained a panel of MAb to surface antigens of SPC cells [1]. This study was aimed at investigating the possibility of using these MAb for *in vitro* immunodiagnosis of metastases of SPC to the bone marrow by indirect immunofluorescence.

MATERIALS AND METHODS

A puncture specimen of bone marrow (3-4 ml) was collected in a tube with 3 ml Eagle's medium with 1% HEPES (Sigma) and 1% heparin (Reanal).

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

Indirect immunofluorescence was carried out with a fraction of live bone marrow nuclear cells adherent to poly-L-lysine (Serva). Nuclear cells from the bone marrow puncture specimen were isolated by centrifugation in a single-step Ficoll-Verografin gradient (density 1.09, Sigma). Hybridoma supernatants were used as the first antibodies, FITC-labeled rabbit polyclonal antibodies to total murine immunoglobulins diluted 1:100 (Sigma) or 1:20 (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences) as the second antibodies. The preparations were examined and photographed in phase contrast and in blue-violet light at wavelength 360 nm under an Opton microscope. In parallel with this, routine cytomorphological diagnosis of metastases was carried out in bone marrow preparations from the same patients.

Cryostat slices of healthy and tumor tissues were incubated with antibodies after an analogous protocol.

RESULTS

Fifty-eight bone marrow puncture specimens collected from different patients prior to chemotherapy were examined. Cytological analysis revealed metastases to the bone marrow in 6 (10.3%) patients.

0007-4888/96/0004-0416$15.00

The presence of cell aggregates of a characteristic morphology in bone marrow preparations (smears) was the criterion of metastases.

Immunofluorescence revealed positive cells (cells with a specific membrane fluorescence) in 12 (20.6%) of the 58 bone marrow preparations. Positive cells were detected in 5 out of 6 cytologically positive cases and in 7 cytologically negative preparations.

The bone marrow specimens positive in terms of the immunofluorescent analysis could be divided into 2 groups. In 7 out of 12 cases positive cells were present mainly as cell aggregates of irregular shape and different densities (Fig. 1). The intensity of staining of different preparations varied. In addition, there were solitary cells in the preparations. In all of these 7 preparations, positive cells stained intensively with antibody H417.1. Contrary to this, other antibodies stained the cells in just a few preparations. Antibody H417.3 stained the cells in 2 preparations, antibody H417.10 in 3, and antibody H417.17 in only 1 out of 7 preparations. Antibody H417.21 did not stain the cells in any of the preparations. Table 1 presents the results of staining of these 7 preparations (group I, preparations Nos. 1-7).

In the remaining 5 positive specimens of bone marrow the positive cells did not form aggregates and were solitary (Fig. 2). In contrast to the above cases, here we did not observe preferential staining of the cells with any particular antibodies. The results of staining of these 5 preparations are presented in Table 1 (group II, preparations Nos. 8-12).

Routine cytomorphological diagnosis revealed metastases to the bone marrow in 10 to 30% of patients with SPC [7,15]. Use of MAb to surface antigens of SPC cells disclosed metastases to the bone marrow in 20-50% of patients in whom they had not been detected by cytomorphological investigation [8,11]. Moreover, micrometastases to the bone marrow were found in 45-55% of patients with localized disease [12]. The presence of positive cells in aspiration biopsy specimens of the bone marrow in these patients was found to correlate with a shorter life span [12]. Hence, the presence of metastases to the bone marrow becomes an extremely important factor for assessing disease dissemination.

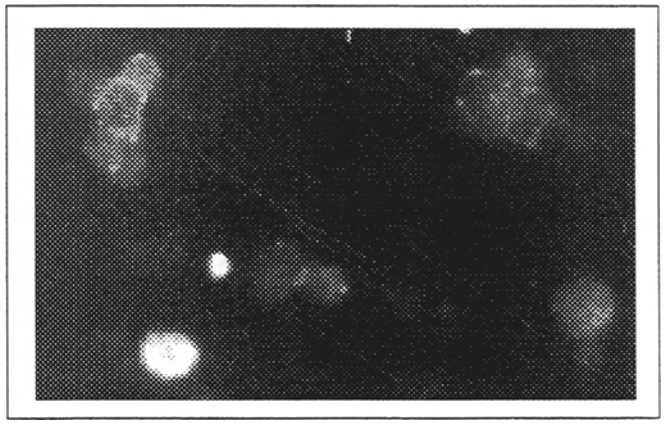

Fig. 1. Aggregates of small-cell pulmonary carcinoma cells, stained with MAb H417.1 (preparation No. 3), in the bone marrow. Indirect immunofluorescence, ×3000.

Because of the cross-reactivity of many antibodies to SPC cell antigens with normal bone marrow cells, specifically, with natural killers and macrophages, additional criteria of the tumorous nature of positive cells in bone marrow preparations from patients are needed, one possible criterion being the presence

TABLE 1. Reaction of Antibodies with Bone Marrow (BM) Preparations from Patients with Small-Cell Pulmonary Carcinoma

BM preparation No.	Monoclonal antibodies				
	H417.1	H417.3	H417.10	H417.17	H417.21
Group I					
1. cytologically positive	+	+	+	-	-
2. -"-	+	+	-	-	-
3. -"-	+	-	-	-	-
4. -"-	+	-	-	-	-
5. -"-	+	-	-	-	-
6. cytologically negative	+	-	+	+	-
7. -"-	+	-	+	-	-
Group II					
8. cytologically negative	-	-	+	-	-
9. -"-	+	+	-	-	+
10. -"-	-	-	+	+	-
11. -"-	-	-	-	+	-
12. -"-	+	+	+	-	-

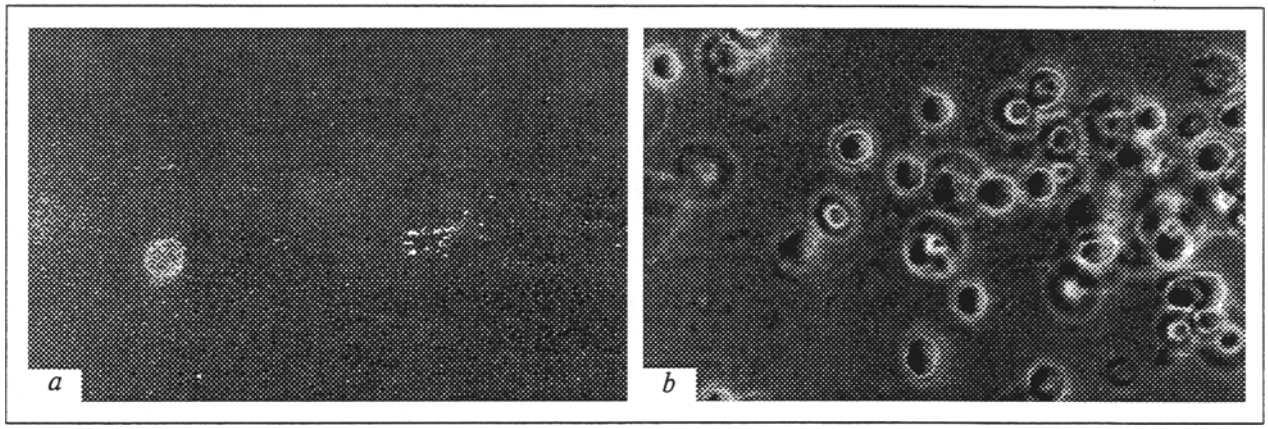

Fig. 2. Solitary positive cell in the bone marrow. Stained with MAb H417.17 (preparation No. 11). ×3000. *a*) indirect immunofluorescence; *b*) phase–contrast image of the same visual field.

of positive cells in the form of rosette aggregates characteristic of SPC [13].

In the first group of positive preparations positive cells presented as rosette aggregates of characteristic morphology and hence, they really were metastatic tumor cells. The nature of positive cells in the preparations of the second group cannot be interpreted so unequivocally. Hence, using MAb, we revealed metastases to the bone marrow in 7 (12.6%) out of 58 cases.

There are published reports describing similar cases, where the diagnosis making use of antibodies is no more sensitive than the routine cytological diagnosis, even with a panel of MAb [13]. This is due to the gradual loss of antigens in metastases [14]. True, our antibodies, especially H417.3, H417.10, and H417.17 stained only a small proportion of the positive preparations, and antibody H417.21 stained none of the positive preparations in group I. Hence, the slight (in comparison with routine diagnosis) improvement of the sensitivity of immunodiagnosis using our MAbs was evidently due to the extreme heterogeneity of expression of the detected antigens in SPC metastases to the bone marrow.

REFERENCES

1. M. A. Goncharskaya, N. M. Rutkevich, A. G. Tonevitskii, *et al.*, *Byull. Eksp. Biol. Med.*, **112**, No. 9, 282-285 (1991).
2. N. I. Perevodchikova an M. B. Bychkov, *Small-Cell Pulmonary Carcinoma* [in Russian], Moscow (1984).
3. L. K. Beck, M. A. Kane, and P. A. Bunn, *Semin. Oncol.*, **3**, No. 2, 300-313 (1988).
4. H. H. Berendsen, L. de Leij, P. E. Postmus, *et al.*, *J. Clin. Pathol.*, **41**, No. 3, 273-276 (1988).
5. P. C. L. Beverley, Y. Olabiran, J. A. Ledermann, *et al.*, *Br. J. Cancer*, **63**, Suppl. 14, 10-19 (1991).
6. P. C. L. Beverley, R. L. Souhami, and L. Bobrow, *Lung Cancer*, **4**, No. 1-2, 15-36 (1988).
7. W. R. Bezwoda, D. Lewis, and N. Livinu, *Cancer*, **58**, No. 8, 1762-1765 (1986).
8. J.-L. Canon, Y. Humblet, A.-M. Lebacq-Verheyden, *et al.*, *Eur. J. Cancer Clin. Oncol.*, **24**, No. 2, 147-150 (1988).
9. F. G. Hay, A. Ford, and C. F. Leonard, *Int. J. Cancer*, **2**, No. 1, 8-10 (1988).
10. F. Hirsch, H. H. Hansen, P. Dombernovsky, and B. Hainau, *Cancer*, **39**, No. 6, 2563-2567 (1977).
11. F. Pasini, G. Pelosi, J. A. Ledermann, and G. L. Cetto, *Int. J. Cancer*, Suppl., No. 8, 53-56 (1994).
12. F. Pasini, G. Pelosi, F. Panavel, *et al.*, *Ann Oncol.*, **5**, Suppl. 8, 161-165 (1994).
13. B. G. Skov, F. R. Hirsch, and L. Bobrow, *Br. J. Cancer*, **65**, No. 4, 593-596 (1992).
14. B. G. Skov, F. R. Hirsch, F. G. Hay, *et al.*, *Ibid.*, **63**, Suppl. 14, 46-48 (1991).
15. D. B. Tritz, D. C. Doll, Q. S. Ringenberg, *et al.*, *Cancer*, **63**, No. 4, 763-766 (1989).