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Tumor-infiltrating lymphocytes expressing IOT-10 marker. An immunohistochemical study of a series of 185 brain tumors

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Summary. The presence of IOT-10-positive lymphocytes among the tumor-infiltrating-lymphocyte (TIL) population was studied in a series of 185 brain tumors. In most of the tumors, IOT-10-positive lymphocytes were identified, but generally they were scarce and masked among the tumor cells, suggesting that NK-cells exercise a poor participation in the tissular response against brain tumors. Isolated tumor cells showing IOT-10-positivity were found in low-grade astrocytomas, neurinomas and medulloblastomas. IOT-10-positivity on both tumor neuropil and tumor cells was considered a characteristic finding in oligodendrogliomas. The number of IOT-10-positive NK-cells in brain metastases and in cerebellar hemangioblastomas was comparatively greater than in other types of brain tumor. Since in brain metastases, the presence of IOT-10-positive NK-cells can be related to the tissular response to an extracerebral malignancy, their considerable presence in cerebellar hemangioblastomas is an enigmatic finding that deserves further attention.

Key words: Tumor-infiltrating-lymphocytes, Immunohistochemistry, IOT-10 marker, NK-cells, Brain tumors

Introduction

In recent years, a great number of details about the lymphocytic cells infiltrating brain tumors have been recorded (Ridley and Cavanagh, 1971; Maunoury et al., 1975; Stavrou et al., 1977; Palma et al., 1978; Phillips et al., 1982; Von Hanwehr et al., 1984; Safdari et al., 1985; Paine et al., 1986; Ullen et al., 1986; Bhondeley et al., 1988; Hitchcock and Morris, 1988; Sawamura et al., 1988; Stevens et al., 1988; Vaquero et al., 1989a, 1990a,b, 1991a), and these data have led to the planning of immunotherapeutic strategies in some types of malignant gliomas (Vaquero et al., 1989b, 1990c, 1991b).

At present, it is well known that NK-cells are a subtype of large granular lymphocytes, which differ from the cytotoxic T-lymphocytes in that their cytotoxic activity is non-restricted by the major histocompatibility complex (MHC). These cells can be recognized immunohistochemically because they express antigens such as Leu-7, Leu-11, CD-16, HNK-1 or IOT-10 (Trinchieri and Perussia, 1984; Vincent and Thiery, 1984). In some circumstances, NK-cells are able to kill tumor cells, and their functional activity can be increased by means of diverse cytokines (Djeu et al., 1979; Herberman et al., 1979; Trinchieri and Perussia, 1984; Vaquero et al., 1990c; Lapeña et al., 1991), suggesting the possibility of therapeutic manipulation should it prove that a greater or lesser number of such cells in the tumor-infiltrating lymphocyte (TIL) population could influence tumor outcome.

In spite of the hypothetical interest in attaining a better knowledge about the biological significance of the presence of NK-cells in tumor tissue, the studies on this subject are scarce, perhaps because of the difficulty of carrying out an immunohistochemical study of these cells from paraffin-embedded material.

For the purpose of evaluating the relative presence of NK-cells participating in the TIL population of brain tumors, we have immunostained the IOT-10 antigen in a series of 185 brain neoplasms. The data obtained are summarized in the present report.

Materials and methods

The present study was performed using paraffin-embedded material from 185 brain tumors (59 astrocytic tumors, 2 ependymomas, 26 meningiomas, 13 neurinomas, 46 brain metastases, 9 primary germinomas, 12 oligodendrogliomas, 14 medulloblastomas and 4 cerebellar hemangioblastomas). From each tumor, two consecutive sections were obtained; one section was processed for haematoxylin-eosin stain, for the purpose of diagnostic confirmation and study of the degree of

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lymphocytic infiltration, and the other was processed for immunohistochemical demonstration of the IOT-10 marker, using the avidin-biotin-peroxidase complex (ABC). Specific murine monoclonal antibody (Immunotech, Marseille) diluted 1:50 was used. After sections were dewaxed they were hydrated with a graded alcohol series and rinsed for 5 minutes in distilled water. After this, sections were incubated for 30 minutes in 0.3% H_2O_2 in methanol, and washed in phosphate-buffered saline (PBS) for 20 minutes. Normal horse serum (3%) was applied for 20 minutes to decrease nonspecific background staining, followed by incubation with primary antibody for 30 minutes in a humidified chamber. This was followed by washing in PBS for 15 minutes, repeated three times. Secondary biotinylated horse antibody against mouse immunoglobulins (Vector Lab., Burlingame, CA) was then applied for 20 minutes, followed by a 45-minute PBS wash. Avidin-peroxidase complex (ABC reagent, Vectastain, Vector Lab., Burlingame, CA) was added for 20 minutes followed by washing for 60 minutes in four 15-minute intervals. 3-amino-9-ethylcarbazole (AEC) was added for 5 minutes, followed by a 5-minute wash, and finally haematoxylin was added for 2 minutes. The slices were mounted with aqueous mounting medium, and observed under the microscope. Positive controls consisted of human tonsil, and negative controls were obtained by incubating the tumor slices with normal mouse serum diluted 1:50 with PBS instead of the primary antibody.

The presence or absence of these cells was classified as: (0) absence of immunostained cells; (+) from 1 to 5 immunostained cells for every 10 microscopic fields, at x 400; (++) from 5 to 10 immunostained cells; and (+++) more than 10 immunostained cells. A minimum of 100 microscopic fields were studied for each tumor. Table 1 shows the histological diagnosis of the tumors in the series, and the patterns of IOT-10 positivity found on the lymphocytes infiltrating the different tumors.

Table 1. Pattern of IOT-10 positivity on lymphocytes infiltrating the different tumors of the series.

TYPE OF TUMOR AND NUMBER OF CASES IN THE SERIES	PERCENTAGE OF CASES SHOWING EACH PATTERN OF IOT-10-POSITIVE LYMPHOCYTES			
	(0)	(+)	(++)	(+++)
Astrocytic tumors (59)				
Low-grade astrocytomas (20)	80%	20%	-	-
Anaplastic astrocytomas (22)	50%	50%	-	-
Multiform glioblastomas (17)	59%	41%	-	-
Ependymomas (2)	-	100%	-	-
Meningiomas (26)	27%	69%	4%	-
Neurinomas (13)	54%	46%	-	-
Metastases (46)	-	57%	41%	2%
Germinomas (9)	-	89%	11%	-
Oligodendrogliomas* (12)	58%	42%	-	-
Medulloblastomas* (14)	36%	57%	7%	-
Hemangioblastomas (4)	-	-	-	100%

(0): absence of immunostained lymphocytes; (+): from 1 to 5 immunostained lymphocytes for every 10 microscopic fields, at x 400; (++): from 5 to 10 immunostained lymphocytes; (+++): more than 10 immunostained lymphocytes. From each tumor, a minimum of 100 microscopic fields were studied. *: In these neoplasms, a significant number of tumor cells expressing the IOT-10 markers were found.

Results

Astrocytic tumors

Among the 59 astrocytic tumors that were studied, 20 cases were classified as low-grade astrocytomas, 22 were anaplastic astrocytomas, and 17 were classified as glioblastomas.

In low-grade astrocytomas, IOT-10-positive lymphocytes were only found in 4 cases (20%). These immunostained cells were always scarce and scattered among the tumor cells (Fig. 1). Their presence was not related to the presence or number of IOT-10-negative lymphocytes in the cases which showed significant lymphocytic infiltration (14 cases). Occasionally, immunostained cells, with features suggesting their tumor nature and a light stain on tumor neuropil, were found in low-grade astrocytomas.

In the anaplastic astrocytomas and glioblastomas, the pattern of IOT-10-positive lymphocytes was similar. These immunostained cells were present in 50% of the anaplastic astrocytomas, and in 41% of the glioblastomas, and were always scarce and scattered as isolated cells among the tumor cells (Fig. 2). In spite of the fact than in 99% of anaplastic astrocytomas and in 55% of the glioblastomas, a significant number of TIL were noted, the number and distribution of IOT-10 positive lymphocytes was not related to the number and distribution of IOT-10-negative lymphocytes.

Ependymomas

The two ependymomas that were included in the present series showed a pattern of positivity similar to that observed in astrocytic tumors; that is, a scarce number of immunostained lymphocytes, scattered among the tumor cells (Fig. 3).

Meningiomas

The present series included 26 meningiomas. In 19 of them (73%), IOT-10-positive lymphocytes were found within the tumor stroma, generally scattered among the tumor cells (Fig. 4). In most of the tumors, a variable lymphocytic infiltration was noted, but the presence and distribution of IOT-10-positive lymphocytes was not related to the number and distribution of IOT-10 negative lymphocytes. The histological subtype of the meningiomas was considered nonrelated to the number of IOT-10-positive lymphocytes.

Neurinomas

We have studied 13 neurinomas in the series. Immunostained lymphocytes were found among the

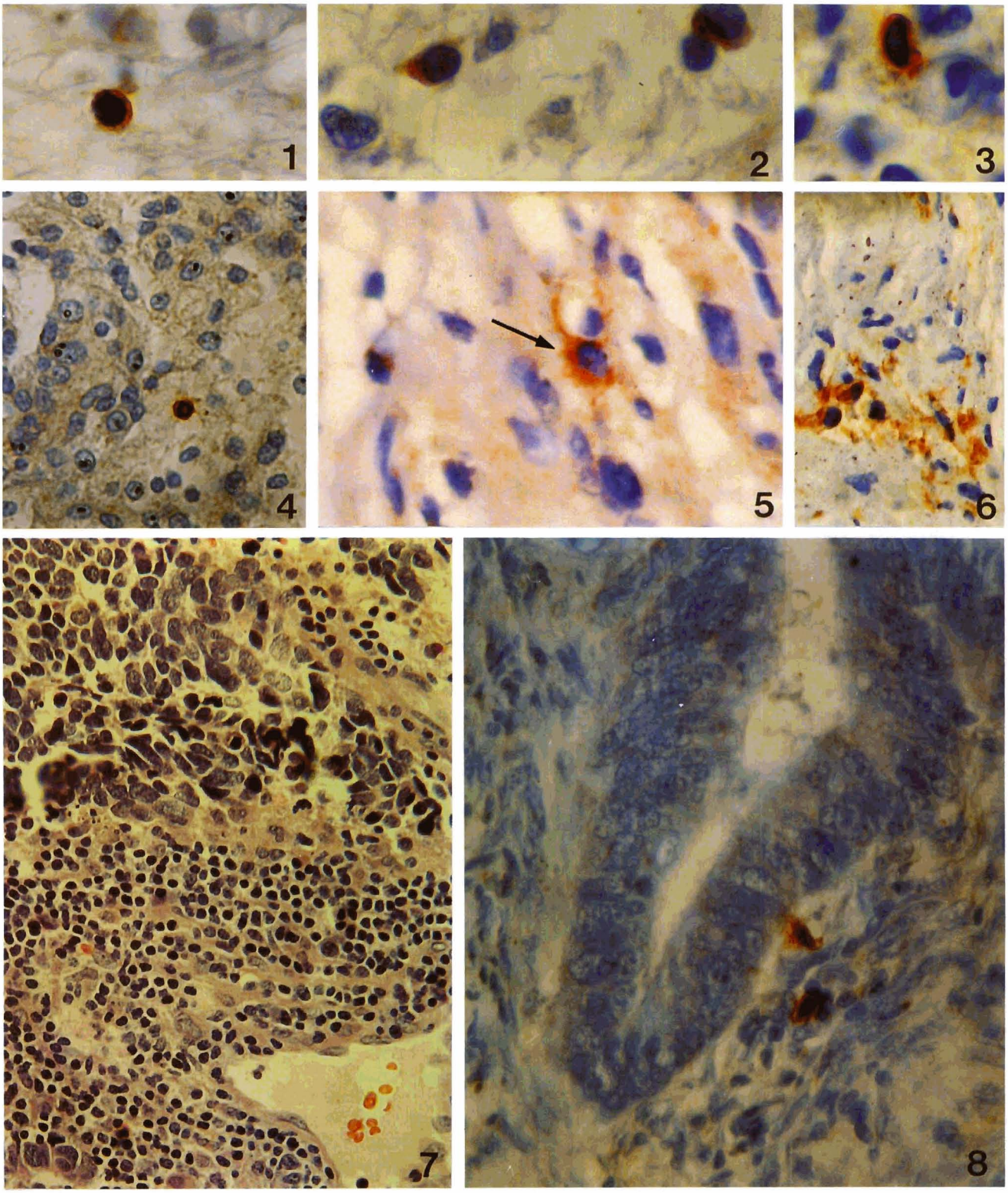


Fig. 1. IOT-10-positive lymphocyte within the stroma of a low-grade astrocytoma. x 400

Fig. 2. IOT-10-positive lymphocytes among tumor cells in an anaplastic astrocytoma. x 400

Fig. 3. Immunostained lymphocyte in an ependymoma of the series. x 400

Fig. 4. Isolated IOT-10-positive lymphocyte in a meningothelial meningioma. x 200

Fig. 5. Intracranial neurinoma. A possible tumor cell, showing positivity for IOT-10 marker in both cytoplasm and cell process (arrow), can be seen. x 400

Fig. 6. Intracranial neurinoma. A granular neuropilic immunostain can be seen. x 200

Fig. 7. Brain metastasis. On haematoxylin-eosin stain, the presence of a great number of TIL is a characteristic finding. x 175.

Fig. 8. Brain metastasis. Two immunostained lymphocytes near the tumor cells can be seen. x 200

tumor cells in only 6 cases (46%). Occasionally, some tumor cells showed immunostained processes, and in some areas, a granular neuropilic stain was observed (Figs. 5, 6).

Cerebral metastases

In the 46 cases that were studied, IOT-10-positive lymphocytes were identified in the tumor stroma, generally in contact with tumor cells. Although in all the cases, a great number of TIL were found (Fig. 7), the number and distribution of IOT-10-positive lymphocytes was not related to the number and distribution of IOT-10-negative lymphocytes (Fig. 8).

Intracranial primary germinomas

Nine cases were studied. In all the cases, IOT-10-positive lymphocytes were identified among tumor cells. Although abundant TIL is a characteristic feature of these tumors, immunostained cells were generally scarce in our material, and their distribution was considered non-related to the distribution of IOT-10-negative lymphocytes.

Oligodendrogliomas

12 oligodendrogliomas were studied in the present series. In at least 5 cases (42%), scarce and isolated IOT-10-positive lymphocytes were identified in the tumor stroma, without apparent relation to the degree or distribution of IOT-10-negative cells (Fig. 9). In at least 11 cases, (92%) a significant immunostain was observed on a great number of tumor cells and on tumor neuropil (Fig. 10).

Medulloblastomas

The present series included 14 medulloblastomas. In 9 cases (64%), isolated IOT-10-immunostained cells, of lymphocytic aspect, were identified among tumor cells (Fig. 11). Nevertheless, in most of the tumors, non-lymphocytic cells showing IOT-10-positivity were noted. This finding was more evident in clear areas of desmoplastic medulloblastomas, that showed clusters of tumor cells with IOT-10 positivity (Fig. 12).

Hemangioblastomas

The 4 hemangioblastomas of the series showed a great number of IOT-10-positive lymphocytes within the tumor stroma and within the blood vessels. Although all the cases showed a significant number of non-IOT-10-positive lymphocytes, the number and distribution of IOT-10-positive cells was considered non related to the pattern of presence and distribution of IOT-10-negative lymphocytes (Figs. 13, 14).

Discussion

In the present work, we have studied the presence of IOT-10-positive lymphocytes within the stroma of 185 brain tumors. The obtained data suggest that the spontaneous presence of IOT-10-immunostained cells in brain tumors is scarce. Generally, IOT-10-positive lymphocytes were masked among tumor cells, and their presence seemed non-related to the degree and distribution of IOT-10-negative lymphocytes. Considering that IOT-10 has been confirmed as a useful marker for identification of NK-cells, our results suggest a poor participation of these cells in the biological mechanisms against tumor tissue, at least when brain tumors are considered. IOT-10-positive lymphocytes were present in significant numbers in only two types of brain tumors that we studied; intracerebral metastases and cerebellar hemangioblastomas. In the case of brain metastases, these findings can be related to the great number of TIL associated with these neoplasms, and agree with the well known tissular response of the brain to the presence of an extracerebral tumor (Vaquero et al., 1990a). In any case, in brain metastases, in a way similar to other intracranial tumors, there exists a discordance between the number of TIL and the number of IOT-10-positive lymphocytes.

In our four cerebellar hemangioblastomas, we have found a great number of IOT-10-positive lymphocytes within the TIL population, a fact which deserves further study for clarification of the biological significance of NK-cell infiltration in this particular type of neoplasm.

Although in the present study the lymphocytic nature of the immunostained cells was recognized in most cases, images suggesting the immunostain of tumor cells were occasionally found in low-grade astrocytomas, neurinomas and medulloblastomas. It was particularly evident in oligodendrogliomas, in which the presence of IOT-10 positivity on tumor cells and within the tumor neuropil was considered a characteristic finding. We explain this finding as being the consequence of the relation between the IOT-10 marker and the HNK-1 epitope that has been recognized in both NK and oligo-

Fig. 9. Oligodendroglioma. A IOT-10-positive lymphocyte and a light neuropilic stain can be seen. x 400

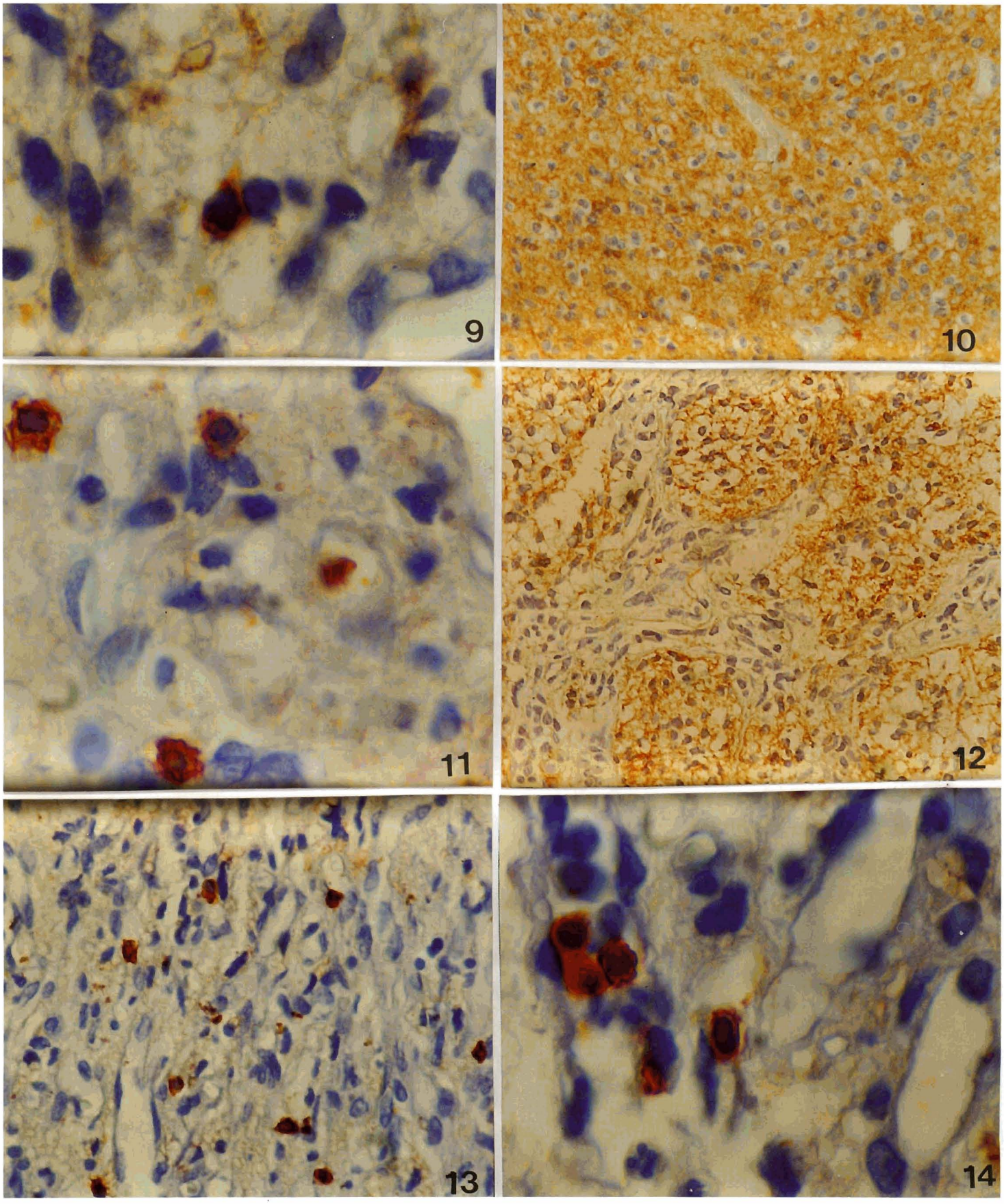
Fig. 10. Oligodendroglioma showing the characteristic IOT-10-positivity in both the tumor cells and tumor neuropil. x 200

Fig. 11. Medulloblastoma. In this field, four immunostained cells can be seen. x 400

Fig. 12. Medulloblastoma showing IOT-10-positivity in tumor cells, mainly in the clear areas. x 200

Fig. 13. Cerebellar hemangioblastoma. A great number of immunostained lymphocytes can be seen. x 200

Fig. 14. Detail of the immunostained lymphocytes, in a cerebellar hemangioblastoma. x 400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dendroglioma cells (Reifenberger et al., 1987).

In any case, we think that our study represents a valid approximation to the knowledge of the patterns of relative presence and distribution of NK-cells in brain tumors. The data that we have obtained here, in a large series of brain tumors, agree with previous observations (Vaquero et al., 1989a, 1990a,b, 1991a) and with the descriptions of other authors using Leu-7 and Leu-11 as immunohistochemical markers of NK-cells (Stevens et al., 1988). On the other hand, the characteristic IOT-10-positivity shown by oligodendrogliomas, suggests the usefulness of this marker, easy to use in paraffin-embedded material, when a differential diagnosis between oligodendroglioma or other tumors with a honey-comb pattern is being considered.

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