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Immunocytochemical study of the cellular immune response in meningiomas

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SUMMARY Twenty four meningiomas (17 benign and seven "atypical" were reacted with a panel of monoclonal antibodies to macrophages, lymphocytes, and HLA DR antigens. All the tumours contained macrophages but these cells were more numerous in the atypical meningiomas. Lymphocytes, almost exclusively of the CD8 subtype, were also present in 70% of benign meningiomas and in all atypical meningiomas and were more abundant in the latter. B lymphocytes were present in minimal numbers in three atypical meningiomas and in one benign meningioma. CD4 positive T lymphocytes were present in small numbers in one benign meningioma and in moderate numbers in one atypical meningioma. HLA DR antigen expression on tumour cells was present in about 60% of both tumour groups.

The numbers of macrophages and T and CD8 lymphocytes in meningiomas seem to be related to atypical histological features, and the presence of these cells raises questions about host immune response and the relation of this to prognosis.

Mononuclear cells infiltrating tumours in man have been noted for at least a century[1] but only lately has attention also been focused on tumours of the central nervous system.[2–11] Very few reports dealing specifically with these cells in meningiomas have been published.

Some patients with meningiomas have a depressed systemic cellular immune response[12 13]; others possess mononuclear white cells which are cytotoxic for their tumours.[14–16] In one malignant meningioma studied in situ with antibodies to T cell subsets[10] and in a few benign meningiomas mononuclear inflammatory cells have been shown.[11 17]

The present study examines the nature and extent of the mononuclear cell infiltrate in benign meningiomas and atypical meningiomas[18] and gives information on the cellular immunity of these tumours.

Material and methods

Snap frozen tissue from 24 consecutive surgically excised meningiomas was available for study. Meningioma was diagnosed and categorised into benign or "atypical"[18] on the basis of paraffin embedded sections stained wit'ı haematoxylin and eosin.

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The frozen tissue from the tumours was stored at −70°C until used and 7 μm cryostat sections were cut and reacted with a panel of antibodies against lymphocyte subsets, macrophages and HLA DR antigens (table 1).

Seven of the meningiomas were "atypical"—that is, they presented a high mitotic rate and at least one of the following histological findings: high cellularity, atypical mitosis, necrosis, and poor differentiation.[18] Clear evidence of invasion of brain in the surgical specimen or clinical evidence of metastasis to extracranial organs were not present but two of the tumours were recurrences eight and 12 years after the first operation when they had shown atypical cytological features.

Of the 17 histologically benign meningiomas, 12 were of the transitional type, three of the fibroblastic type, one was meningotheliomatous and one haemangiopericytic. Frozen tissue blocks from the tumours were selected and the sections cut measured about 1 square cm. Monoclonal antibody reactions were carried out using the indirect two stage immunoperoxidase technique.[19] The sections studied did not show gross necrosis of the tumours. The approximate number and distribution of positive cells in the tumours were examined looking both at areas of tumour parenchyma (away from blood vessels) and

Table 1 *Antibodies used*

Monoclonal antibody (dilution)	*Isotype*	*Specificity*	*Source/reference*
RFD7 (1:1)	IgG1	Macrophages	G Janossy, Royal Free Hospital
Y182A (1:1)	IgG3	Macrophages	K Gatter, Oxford
RFDR1 (1:1)	IgM	HLA DR	G Janossy, Royal Free Hospital
VICY1 (1:100)	IgG1	HLA DR invariant chain	W Knapp, Vienna
RFBCT (1:1)	Cocktail IgM (CD20) IgG (CD20) RFBT	B lymphocytes	G Janossy, Royal Free Hospital
RFTCT (1:1)	Cocktail IgG (CD2) IgG (CD3) IgG (CD7) IgG (CD8Y)	T lymphocytes	G Janossy, Royal Free Hospital
T8 (1:10)	IgG1	T suppressor/cytotoxic	Dako
T4 (1:10)	IgG 1K	T helper/inducer	Dako

perivascular spaces. Macrophages in the latter compartment were subdivided into perivascular and "pericytal" (defined as cells distinctly seen wrapped around blood vessels in a pericytal pattern). The density of positive cells in the various areas was assessed using a scale from 1 to 4 where 1 indicated occasional positive cells in a high power field (× 400); 2 up to 20 cells; 3 from 20 to 40 cells; and 4 more than 40 cells.

Results

The results obtained with the antibodies RFD7, Y182A, RFTCT and T8 are given in table 2 which summarises results for atypical meningiomas and benign meningiomas separately.

In benign meningiomas the antibody RFD7 showed reactive cells (macrophages) in 76% of cases and Y182A in 93% within tumour parenchyma. More reactive cells (macrophages) were detected with Y182A (average cellularity 1·8) than RFD7 (average cellularity 1·4). Reactive cells (macrophages) were seen scattered throughout the tumours (fig 1) as well as in groups. In the same areas these cells formed perivascular collections, but in other areas they were intimately mixed with tumour cells including those forming whorls in meningiomas of the transitional type.

In atypical meningiomas macrophages were present in all cases with both antibodies, and the average cellularity was much higher than in benign meningiomas (3 with RFD7 and 3·7 with Y182A). Within

Table 2 *Meningiomas (benign and "atypical")*

No of cases	*Site*	*Semiquantitative rating*					*Mean rate*	*Percentage of positive cases*
		0	*1*	*2*	*3*	*4*		
RFD7								
17	Cellularity in tumour	4	4	6	3		1·4	76
7	proper			2	3	2	3	100
15	Perivascular cellularity	5	6	4			0·9	66
7				2	5		2·7	100
13	"Pericytal" cellularity	9	1	3			0·5	30
7		1	1	3	2		1·8	85
Y182A								
16	Cellularity in tumour	1	1	11	2		1·8	93
7	proper				2	5	3·7	100
13	Perivascular cellularity	2	6	3	2		1·3	84
7				2	5		2·7	100
13	"Pericytal" cellularity	2	7	3	1		1·2	84
7			1	6			1·8	100
RFTCT								
17	Cellularity in tumour	1	11	5			1·2	94
7	proper		1	5	1		2	100
17	Perivascular cellularity	2	5	9	1		1·5	88
7			1	4		2	2·3	100
T8								
17	Cellularity in tumour	5	6	6			1	70
7	proper		2	3	2		2	100
17	Perivascular cellularity	6	7	3	1		0·9	64
7			2	4	1		1·8	100

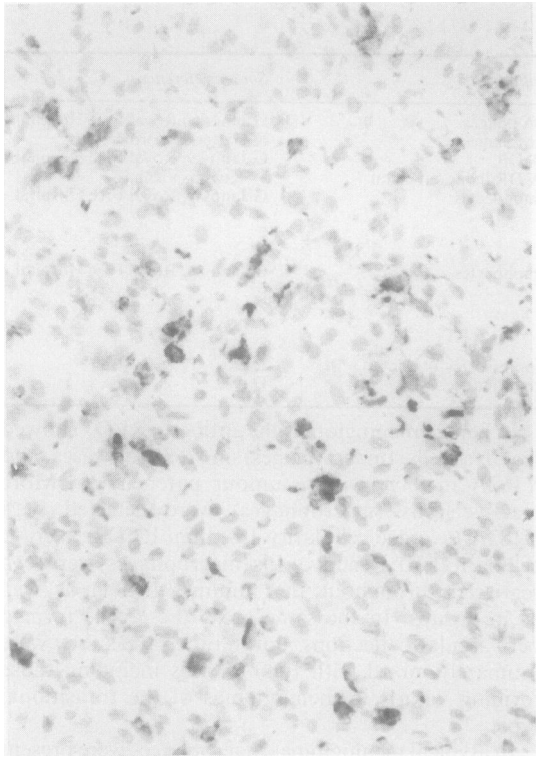

Fig 1 *Benign meningioma: scattered macrophages in parenchyma (RFD7).*

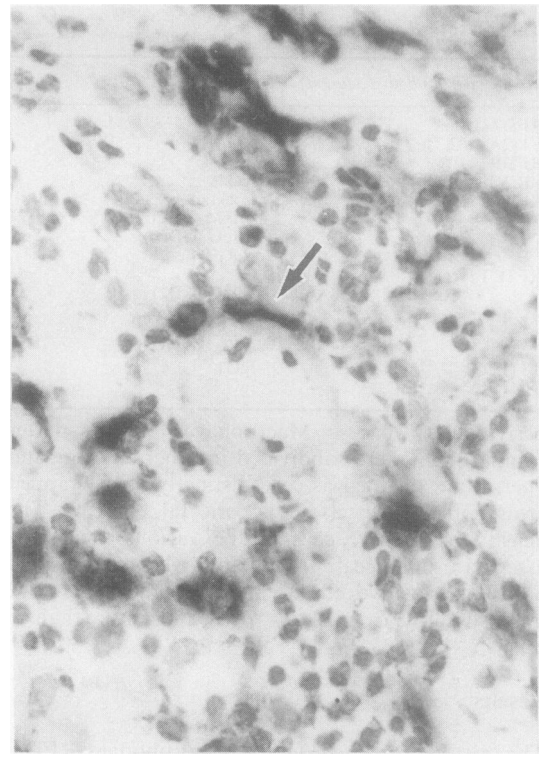

Fig 3 *Atypical meningioma: perivascular macrophages and "pericytal" macrophage (arrow).(Y182A.)*

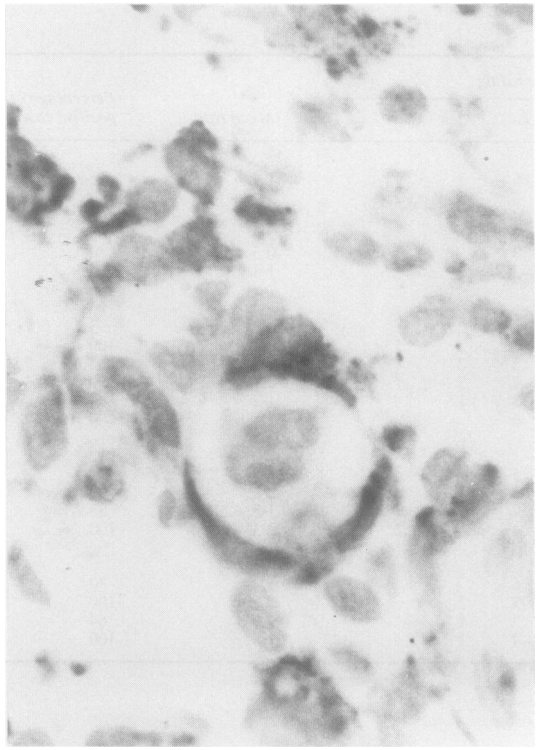

Fig 2 *Atypical meningioma: whorl formation. Note tightly wrapped cells stained with RFD7 (antimacrophage monoclonal antibody).*

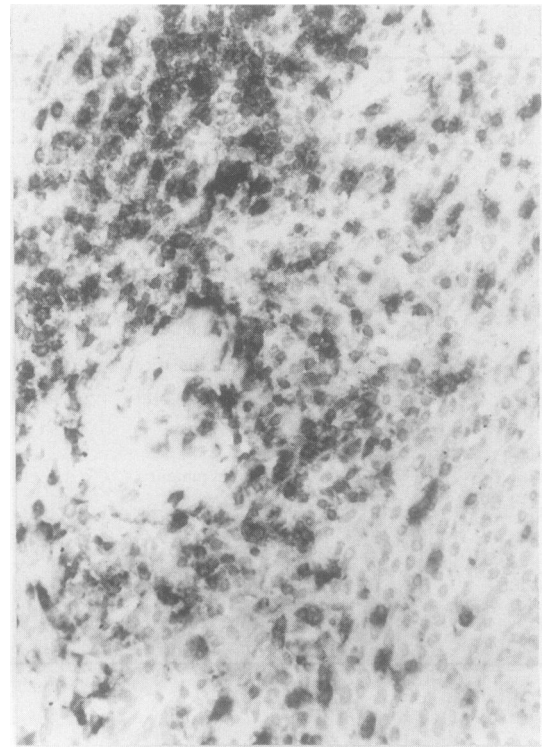

Fig 4 *Atypical meningioma: note oblique blood vessel with large numbers of perivascular and parenchymal T lymphocytes. (RFTCT.)*

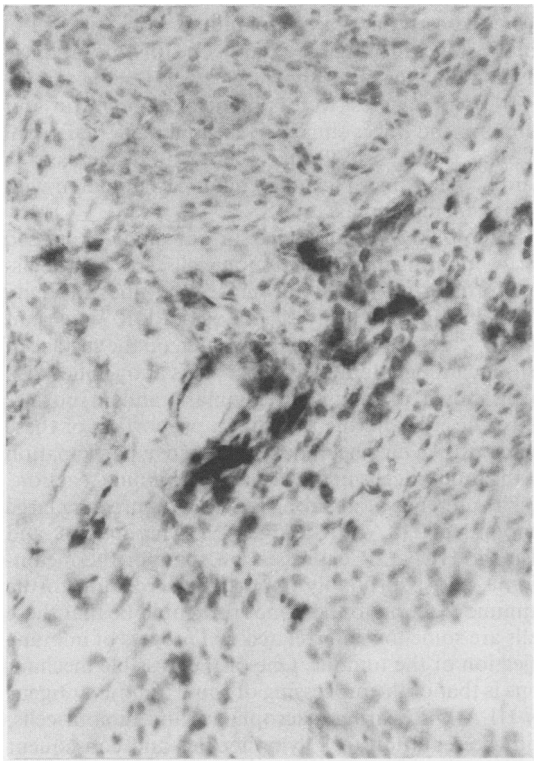

Fig 5 *Benign meningioma: perivascular and parenchymal T8 lymphocytes. (T8.)*

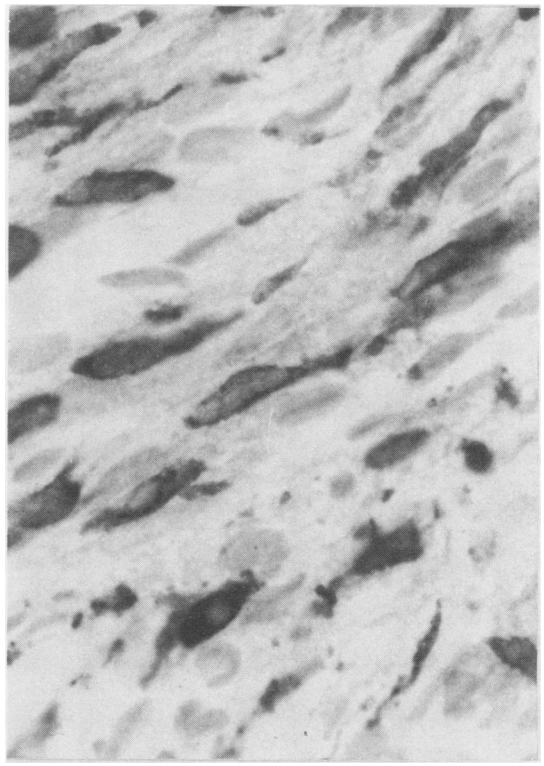

Fig 6 *Benign meningioma: tumour parenchyma with spindle cells interpreted as tumour cells reactive with VICY1 (anti-MHC invariant chain).*

whorls it was difficult on morphological grounds alone to distinguish the cells reactive with the macrophage antibodies from the unreactive tumour cells (fig 2).

In perivascular spaces (fig 3) macrophages were seen in 66% with RFD7 and 84% with Y182A in benign meningiomas, the cellularity being 0·9 and 1·3, respectively. All atypical meningiomas, on the other hand, contained macrophages, and the average cellularity with both antibodies in perivascular spaces was 2·7.

Macrophages in pericytal disposition (fig 3) were seen in 30% of benign meningiomas with RFD7 and in 84% with Y182A, the cellularity being 0·5 and 1·2, respectively. In atypical meningiomas RFD7 showed macrophages in 85% and Y182A in 100%; the cellularity was 1·8 with both antibodies. Macrophages also showed strong positivity in all areas with the HLA DR antibody VICY1 and weaker positivity with RFDR1.

In benign meningiomas T lymphocytes, identified with the antibody cocktail RFTCT, were present in 94% of tumours within parenchyma; all the atypical meningiomas were positive. The cellularity was 1·2 in benign meningiomas and 2 in atypical meningiomas. Atypical meningiomas also showed more T lymphocytes in perivascular spaces (fig 4) than benign meningiomas (cellularity 2·3 *v* 1·5), and in a higher percentage of cases (100% *v* 88%).

T8 positive lymphocytes were distributed within both groups of meningiomas in a pattern similar to that observed with RFTCT within tumour and in perivascular spaces. Benign meningiomas showed positivity in 70% of cases within the tumour (average cellularity 1) and in 64% of cases in perivascular spaces (cellularity 0·9) (fig 5). By contrast, the atypical meningiomas showed T8 lymphocytes in both areas in all cases and the average cellularity was 2 and 1·8, respectively.

T4 lymphocytes were present in minimal numbers in one benign meningioma and in moderate numbers in one atypical meningioma. B lymphocytes were present in very small numbers in three atypical meningiomas and one benign meningioma. HLA DR antigen was detected with the antibody RFDR1 on tumour cells in 64% of benign meningiomas and in 57% of atypical

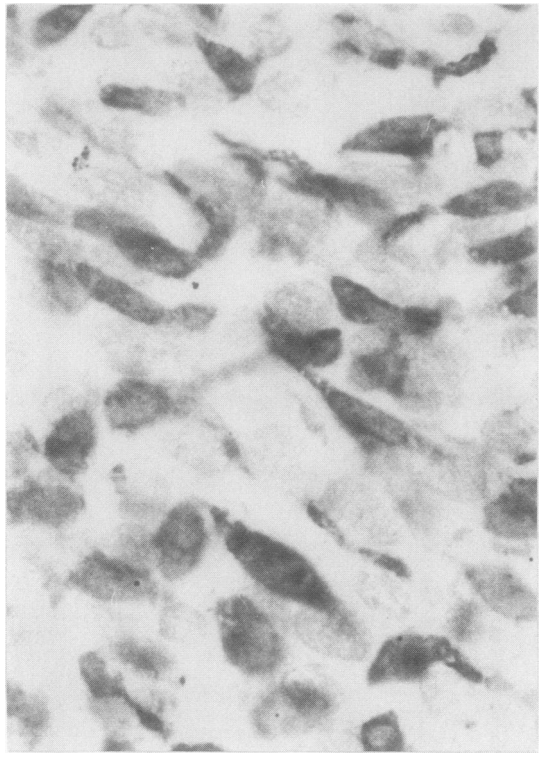

Fig 7 *Atypical meningioma: tumour cells staining for HLA DR as in fig 6. (VICY1.)*

meningiomas. VICY1 showed cytoplasmic positivity in six of nine benign meningiomas (fig 6) and in two of seven atypical meningiomas (fig 7).

Discussion

Previous studies have shown that meningiomas have a large macrophage infiltrate, and in one report these averaged 42% (with a range between 5 and 80%) of the total number of cells obtained from tumour cell suspensions.[11] The clinical importance of these findings is still not clear and whether they are associated with a better prognosis in a particular tumour is not known. A possible interpretation of the higher number of macrophages infiltrating atypical meningiomas is that these reflect a stronger immune response to more aggressive tumours. This could be supported by the finding that two of our atypical meningiomas were recurrences at eight and 12 year intervals.

The absence of frank necrosis in the sections of atypical meningiomas studied suggests that they are not solely concerned with the phagocytosis of cell debris.

T lymphocytes have been shown in meningiomas in a limited series and a predominance of T8 in one of four benign meningiomas and one of one atypical meningioma has been shown.[10]

T4 lymphocytes were practically absent in our tumours and this is similar to the finding in malignant gliomas.[19] We also found a four fold increase in lymphocytes in atypical meningiomas compared with benign meningiomas.

Some of the meningiomas in our study expressed HLA DR antigens within tumour cells. After cytoplasmic processing (stage recognised by the antibody VICY1 directed against the HLADR invariant chain)[20,21] HLA DR antigen is located on the surface of cells including "immune" cells.[22] This antigen has also been shown in malignant gliomas[19] and in normal astrocytes,[23,24] and the fact that a proportion of these were positive may be indicative of active participation of the tumour cells in the process of immune reaction.

The presence of macrophages (also expressing large amounts of HLA DR antigen), T lymphocytes, and also the HLA DR expressed on some of the meningioma cells is probably important in relation to the immune reaction to the tumour. It could be that these cells are somehow interrelated in a process of immune rejection of the tumour. One of the possible mechanisms is that of the processing of some tumour antigens by HLA DR bearing macrophages and tumour cells, with presentation to T lymphocytes and consequent activation of the latter.[25]

As far as we know this study is the first to report that the numbers of macrophages and T and CD8 lymphocytes in meningiomas are related to atypical histological features. The widespread presence of these cells in meningiomas raises questions concerning the host's immune response and the possible relation of this to prognosis. The presence or absence of these infiltrates may help (combined with other histological features) to assess the likely aggressive behaviour of the tumour, although this still has to be supported by long term follow up studies.

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