Expression of macrophage/histiocytic antigens in pleomorphic xanthoastrocytomas

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Pleomorphic xanthoastrocytoma (PXA) is a rare variant of a superficial cerebral astrocytoma characterised by distinct clinical and histological features. Its derivation from subpial astrocytes has been proposed, although the capacity of neoplastic cells for expression of different immunohistochemical markers is still under debate. These immunohistochemical studies were performed on eight cases of PXA in order to evaluate the expression and co-expression of glial and macrophage/histiocytic markers in various tumour cell populations. The expression of antigens was examined with the use of single- and double-immunolabelling methods for GFAP, vimentin, LCA, CD68, HLA-class II and MAC 387. All the cases of PXA showed variable immunoreactivity to GFAP, both in spindle-shaped and pleomorphic lipidised tumour cells. A subset of neoplastic cells was stained strongly with HLA-class II monoclonal antibody and with antibody to CD68. The reactivity to LCA and MAC 387 was absent in neoplastic cells, while it was easily evidenced in the non-neoplastic infiltrative component. The immunohistochemical double staining demonstrated the co-expression of GFAP and HLA-class II or CD68 antigens in the cytoplasm of individual neoplastic cells, including large pleomorphic, lipid-laden ones. It seems that tumour cells in PXAs derived from subpial astrocytes reveal monocytic/macrophage immunophenotype and demonstrate the capability of functional behaviour as mesenchymal cells with phagocytic activities. The variability in expression of antigens related to glial and monocytic/macrophage differentiation stressed the immunophenotypic heterogeneity of tumour cells in PXAs.

key words: pleomorphic xanthoastrocytoma, immunohistochemistry, macrophage/histiocytic antigens

INTRODUCTION

Pleomorphic xanthoastrocytoma (PXA) is a rare variant of astrocytic glioma occurring primarily in childhood and adolescence with distinctive histological and clinical criteria. Since the first such case was documented [21], a number of new reports of this entity have been published [1–3, 11, 13, 15, 18, 35, 38, 39, 43, 44]. PXA usually develops supratentorially in a superficial localisation, often involves the leptomeninges and is accompanied by a cyst. In keeping with its name, the tumour exhibits extreme cellular and nuclear pleomorphism. Other neoplasms with similar pleomorphic morphology, such as glioblastomas [7] and malignant fibrous histiocytomas [12], should be differentiated in diagnosis. In contrast to these malignant pleomorphic...
tumours, PXAs are characterised by a relatively slow clinical course and a favourable prognosis, despite the histological features of nuclear and cellular atypia [5, 21].

Understanding of the pathogenesis of PXA remains incomplete [9]. The current view, originally proposed by Kepes et al. [24], maintains that PXA is most likely derived from subpial astrocytes, which are normally invested by basal lamina and are associated with positive reticulin staining. The majority of immunohistochemical studies have been largely applied to establish the glial nature of tumour cells [24, 48] or to demonstrate limited neuronal differentiation in some cases of PXA [10, 33].

The aim of this study has been to elucidate the immunophenotypic features of PXA connected with monocytic/macrophages antigens. We have presented both single and double immunohistochemical labelling techniques for visualisation of the expression and co-expression of glial and monocytic/macrophages or histiocytic markers.

**MATERIAL AND METHODS**

The study was performed on eight selected cases of PXA that fulfilled the clinical and histological criteria of typical PXA, originally outlined by Kepes et al. [21]. The patients were mostly young (ranging in age from 17 to 56 years) and with histories of epileptic seizure and supratentorial tumours. MRI revealed a well-circumscribed contrast-enhancing mass located superficially in the parietal or temporal lobe and often accompanied by a cyst.

The histopathological studies were performed on biopsy material fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections were routinely stained with hematoxylin and eosin (H & E) and by Gomori’s method. The frozen sections were stained with Oil-Red O for neutral lipids.

Immunohistochemical analyses were performed on paraffin-embedded specimens with polyclonal antibodies against glial fibrillary acidic protein (GFAP), vimentin and with monoclonal antibodies against leukocyte common antigen (LCA), monoclonal anti-human leukocyte antigen class II (HLA-DP, DQ, DR), monoclonal anti-human macrophage antigens — CD68 (clone KP1) and monoclonal anti-human myeloid/histiocyte antigen — MAC 387 (all antigens from Dako). The expression of antigens was examined using single- and double-immunolabelling methods according to the labelled streptavidin-biotin complex method or APAAP methods with DAB or Fast Red as responding chromogens.

**RESULTS**

Histologically, all tumours were highly pleomorphic, composed of polygonal, spindle-shaped, lipid-laden and frequently multinucleated large cells, exhibiting foamy, vacuolated or “xanthomatous” cytoplasm (Fig. 1A). Numerous eosinophilic granular bodies and eosinophilic “hyaline” droplets were seen. Clusters of lymphocytes and/or plasma cells were frequently observed. The histological features of malignancy such as mitoses, endothelial proliferation and necroses were absent. An abundance of reticulin fibres was present, often surrounding the clusters or individual tumour cells. Many bizarre, multinucleated giant cells contained large amounts of neutral lipids, evidenced by selected Oil Red stain.

**Immunohistochemical investigation**

All cases exhibited vimentin and GFAP-immunopositivity in the cytoplasm of many tumour cells, including both small, spindle-shaped cellular components and large, bizarre or multinucleated cells with a multitude of lipid droplets (Fig. 1B). Even the heavily lipidised tumour cells with large lipid droplets occupying much of the cellular body demonstrated the distinct peripheral GFAP expression. However, a number of tumour cells were immunonegative for GFAP.

Immunostaining for monoclonal human leukocyte antigen (HLA) class II (DP, DQ and DR) was strongly positive in a large number of morphologically different neoplastic cells, including large foamy cells. Expression of HLA was seen both on the surface and in the cytoplasm of tumour cells (Fig. 1C). Some large heavily lipidised tumour cells with numerous lipid-droplets exhibited granular staining for HLA (Fig. 1D). HLA was also positive in a moderate number of granular bodies scattered within pleomorphic neoplastic tissue. Focal staining for HLA was also seen in reactive macrophages in PXAs.

A significant number of tumour cells of great variability in size and shape demonstrated strong reactivity to monoclonal monocytic/macrophage associated antibody CD68 (Fig. 1E). The pattern of CD68 immunoreactivity in some lipid-laden tumour cells was similar to GFAP positivity. Some large tumour cells expressed exceptionally strong CD68 immunoreactivity (Fig. 1F), whereas other heavily lipidised tumour cells containing large lipid vacuoles exhibited only discrete CD68 positivity (Fig. 2A). Moreover, the CD68 antibody reacted with the small cellular component associated with monocytic/macrophage infiltrates.

The tumour cells were generally negative for leukocyte common antigen (LCA), whereas scattered lymphocytic or lympho-plasmocytic infiltrations reacted with LCA. Individual tumour cells were only occasionally labelled with LCA.

Immunoreactivity for MAC 387, a highly specific histiocytic marker, was negative in both the giant and spin-
Figure 1. Histological and immunohistochemical features of PXAs. 

A. A typical picture of PXA with a pleomorphic cellular population containing spindle-shaped, large and round lipid-laden multinucleated tumour cells. The presence of numerous eosinophilic granular bodies and clusters of lymphocytes. HE, original magnification × 250; 

B. Strong GFAP immunoreactivity in the cytoplasm of spindle-shaped and pleomorphic lipidised tumour cells; 

C. Diffuse expression of HLA class II seen on the cell surface and in the cytoplasm of numerous tumour cells; 

D. Granular pattern of immunostaining for HLA in the large, heavily lipidised tumour cells and strong reactivity in the other cells; 

E. Positive reaction for CD68 in various cellular components, including lipidised tumour cells; 

F. Strong cytoplasmic reactivity to CD68 in large tumour cells; 

B–F original magnification × 400.
Figure 2. Immunohistochemical features of PXAs. A. Heavily lipidised tumour cells with large lipid vacuoles exhibiting slight CD68 positivity; B. Immunostaining for MAC 387 limited to non-neoplastic, reactive intra- and perivascular monocytes and/or histiocytes; C. Double immunolabelling for GFAP (brown) and HLA (red) demonstrates varied reactivity related to different cellular components; D. Co-expression of GFAP (brown) and HLA (red) in large vacuolated lipid-laden cells; E. Double labelling for GFAP (brown) and CD68 (red) with variability of expression and co-expression; F. Co-expression of GFAP (brown) and CD68 (red) seen in some neoplastic cells; A–F. Original magnification × 400.
dle-shaped tumour cells. However, positive staining for MAC 387 was occasionally demonstrated in small non-neoplastic, reactive monocytes or histiocytes, which, together with lymphocytes, formed inflammatory infiltrates in PXA. The intra- or peri-vascular monocytes and granulocytes were also strongly stained with MAC 387 (Fig. 2B).

Immunohistochemical double labelling with antibodies for GFAP and HLA demonstrated various patterns of immunoreactivity and its co-expression. In some areas the antibodies labelled the different cellular elements; some tumour cells were predominantly stained with GFAP, whereas the other cellular components reacted strongly with HLA (Fig. 2C). The co-expression for GFAP and HLA-class II was evidenced in the cytoplasm of a subset of neoplastic cells of various morphology including large, vacuolated, lipid-laden pleomorphic tumour cells (Fig. 2D). Similar results were obtained with double immunohistochemical staining using antibodies against GFAP and CD68. Many tumour cells exhibited either a different staining of GFAP and CD68 or their co-expression (Fig. 2E). In individual cells double staining revealed distinct red particles of CD68 immunoreactivity on some GFAP immunopositive cell bodies and cytoplasmic processes. Distinct CD68 immunoreactivity was demonstrated in the form of scattered granules within GFAP-positive tumour cells (Fig. 2F).

**DISCUSSION**

PXA is an independent entity of low-grade astrocytic cerebral neoplasm with characteristic pleomorphic histopathology. The key problem in differential diagnosis of PXA is its distinction from other pleomorphic neoplasms of different origin. This neoplasm was at first mistaken for a mesenchymal and not a glial tumour because of its advanced lipidisation and abundance of reticulin fibres [20]. The parts of the tumour containing a number of spindle-shaped neoplastic cells resembling fibroblasts and a dense network of reticulin fibres corresponding to pericellular basement membrane strongly suggested its mesenchymal origin. Moreover, the lipidisation of tumour cells observed in PXA was also typical for malignant histiocytic tumours [20, 32] and some glioblastomas [8, 22]. The overgrading in cases of PXA might be limited by considering the distinct clinical and radiological features of this entity. The correct diagnosis is crucial, because PXA, despite its highly pleomorphic cytology, is characterised by a relatively favourable prognosis and does not require an aggressive postoperative therapy. Its relatively benign clinical behaviour has been confirmed by many authors [1, 11, 13, 27, 31, 38, 47]. Nevertheless, a few reports have indicated more aggressive behaviour and stress the possibility of malignant tumour evolution [3, 4, 14, 23, 46].

The immunohistochemical studies are very helpful in the differential diagnosis of tumours of various origin especially when considering tumours with a light-microscopic pleomorphic morphology [32, 40, 41]. The expression of GFAP establishes the astrocytic derivation of tumour cells in PXA [24] and is of great value in differential diagnosis from GFAP-negative meningeal malignant fibrous histiocytoma [12, 16, 18, 37]. The majority of tumour cells in PXA are considered astrocytic in nature, although recent studies indicate evidence of neuronal differentiation and a relationship between PXA and glioneuronal neoplasm [6, 10, 25, 29, 33, 45].

The present immunohistochemical investigations have revealed the variability of monocyte/macrophage antigen profiles in typical PXA cases. A number of tumour cells were immunopositive for monocytic/macrophage antibody CD68 and HLA class II. The large, lipidised tumour cells characteristic for PXA often express GFAP immunoreactivity with co-expression of HLA and CD68 in the cytoplasm and cellular processes. The CD68 antigen (clone KP1) represents the transmembrane protein localised in lysosomes and is considered a panhistiocytic marker useful in the diagnosis of histiocytic tumours [34]. However, the positive reaction to CD68 was not enough to establish a true histiocytic origin, since it was also observed in tumours of different origin i.e. xanthomatous meningioma [42]. In this study the immunoreactivity for CD68 was evidenced both in many large, pleomorphic tumour cells and in the cells partially related to monocytic and lymphocytic infiltrations. Also the majority of HLA labelled cells ought to be considered tumour cells and only small scattered cells interpreted as part of the reactive macrophage population. In contrast, MAC 387, a marker of histiocytes with a high degree of specificity, was entirely negative in tumour cells but could be seen in reactive histiocytes within inflammatory perivascular infiltrates.

Immunohistochemical double labelling with antibodies to GFAP and CD68 or HLA demonstrated the expression of both the glial and monocytic/macrophage antigens in the same tumour cells of PXA. The co-expression of GFAP with HLA and CD68 in these glial tumours evidences a capability of tumour cells to express the monocytic/macrophage phenotype. The pattern of immunoreactivity strongly emphasised the phenotype heterogeneity of PXA.

The unique histological and immunohistochemical features of PXA might in part result from chronic degenerative and regressive changes in tumour cells represented by the eosinophilic hyaline granules, lipid drop-
lets, and microcystic changes or calcifications [18]. Lipidized neoplastic astrocytes in otherwise malignant glioma are usually found near coagulative necrosis. The lipid deposits in tumour cells are thus often considered to be related to necrobiotic processes. It seems that lipidized astrocytes may mimic the cells of mesenchymal origin by their ability to act as macrophages, particularly in tissue culture [28]. Some other markers of mesenchymal origin by their ability to act as macrophages, particularly lipidised astrocytes may mimic the cells of mesenchymal origin. It seems that lipidised astrocytes reveal the expression of monococyte/macrophage phenotype. This might be related to the peculiar nature of tumour cells associated with the unique spectrum of histological features and heterogenous immunophenotype of PXA.

REFERENCES

4. Chakrabarty A, Mitchell P, Bridges LR, Franks AJ (1999) Macrophage phenotype. It seems that this type of glia might behave as “facultative mesenchymal cells” and demonstrate the capability of functional behaviour as mesenchymal cells with phagocytic activities. This might be related to the peculiar nature of tumour cells associated with the unique spectrum of histological features and heterogenous immunophenotype of PXA.

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