LYMPHOCYTIC INFILTRATION IN GLIOMAS: EVIDENCE OF POSSIBLE HOST RESISTANCE

BY

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INTRODUCTION

During the past fifteen years it has become increasingly evident that immunological processes are an important factor in the host's resistance to the development and subsequent growth of spontaneous tumours in experimental animals (Gorer and Amos, 1956; Prehn and Main, 1957; Old and Boyse, 1964; Burnet, 1969). It is also becoming apparent that a similar situation exists in regard to human tumours (see Fairley, 1969).

It is now realized that certain changes occur in cancer cells which result in their no longer being recognized immunologically by the body as "self." Two processes are involved in this change: one is loss of normal tissue specific antigens ("antigenic deletion"); the other is acquisition of new surface antigens ("tumour specific antigens") not present in normal cells (Gorer, 1956; Klein, 1966). Burnet (1962) has suggested that the fundamental role of "cellular immunity" is to recognize these mutant "non-self" cells and destroy them before they give rise to a tumour. There is a growing body of evidence to support the general correctness of this theory, and for believing that breakdown of this monitoring system in later life may, by allowing tumour cells to gain a foothold, permit a tumour to develop (Burnet, 1967). Once a tumour is established, its growth usually continues to outstrip the inhibitory action of any immunological defence mechanisms directed against it. In most cases the immunological reaction which is mounted against the tumour is relatively feeble (Alexander and Fairley, 1967), but very rarely it may succeed in eradicating the tumour and lead to an apparently spontaneous cure (Everson, 1964). There are two possible reasons for the inherently weak response by the host. First, the central immune mechanisms of those who develop tumours may be impaired (Sparshott and Augustin, 1970) so that an effective rejection response cannot be mounted against the tumour. Secondly, changes in the surface properties of tumour cells may block the afferent limb of the immune response either by reducing the antigenicity of
tumour tissue or by interfering with the peripheral sensitization of lymphocytes (Currie and Bagshawe, 1968; Billington, 1969).

Cellular immune responses are generally believed to be more important in the rejection of solid tumours than humoral antibodies (Alexander and Fairley, 1967), and a hall-mark of the rejection response is invasion of the foreign tissue by lymphoid cells. Lymphocytic infiltration has long been recognized as a prominent histological feature of animal and human tumours (Wade, 1908; MacCarty, 1922; Foote and Stewart, 1946), but only latterly, with clarification of the immunological function of the lymphocyte has this been considered in relation to the growth and fate of the tumour. Following these ideas, Hamlin (1968) made a detailed study of lymphocytic infiltration of cases of mammary carcinoma and clearly demonstrated that the prognosis of the tumour depended as much upon the degree of lymphocytic reaction as upon the grade of malignancy of the cancer.

Lymphocytic infiltration around gliomas has been noted by Bertrand and Mannen (1960) but without consideration of its relevance. In view of the possible importance of this feature as evidence of a host defence mechanism against the tumour we have reviewed post-mortem material from a series of glioma cases to determine the frequency and the degree of lymphocytic infiltration.

**Materials and Methods**

The post-mortem records of all patients dying of cerebral tumour at the London Hospital from 1959 to 1966 were examined. Only cases of glioma were taken for the present study and 93 such cases were found, consisting of 86 astrocytomas (80 cerebral and 6 brain-stem), 2 oligodendro-gliomas, 2 medulloblastomas and 3 ependymomas. Ninety were adults, ranging from 24 to 73 years of age, and 3 were children aged 4, 5, and 12 years. There were 56 males and 37 females. One or more histological slides of the tumour and the adjacent brain, stained with haematoxylin and eosin, were available from the Pathological Institute of the London Hospital. The slides were examined by each of us independently, graded according to the method of Kernohan (Kernohan et al., 1949) and the results collated. At the same time the degree of lymphocytic infiltration either in the form of perivascular cuffing or as diffuse infiltration was described for each tumour and placed into one of three categories, A, B, or C, according to whether the reaction was definite, slight or absent (Table I). A rough quantitative estimate of the degree of lymphocytic reaction in Group A tumours as judged by the number of cuffed vessels and their thickness was made and recorded as + when it was moderate (fig. 1, Plate IX) and ++ when it was marked (fig. 2). The occurrence of plasma cells was also noted.

**Results**

**Group A**

Definite lymphocytic reaction was present in 28 (30 per cent) of the present series, and in 11 of these cases cuffing was marked (++). All 28 cases were adults with cerebral astrocytomas and the commonest site was the temporal lobe (36 per cent). 27 of the tumours were highly malignant (Grades 3 and 4); the remaining tumour was a gemistocytic astrocytoma (Grade 2). The high malignancy of this group was reflected in the short clinical histories of most of the cases. Unfortunately,
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Table I

<table>
<thead>
<tr>
<th>Lymphocytic reaction (in and/or around tumour)</th>
<th>Group A Definite</th>
<th>Group B Slight</th>
<th>Group C Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases (Total 93)</td>
<td>28 (30%)*</td>
<td>26 (28%)</td>
<td>39 (42%)</td>
</tr>
<tr>
<td>Average age (Years)</td>
<td>57 (24-73)†</td>
<td>40 (12-73)</td>
<td>43 (4-68)</td>
</tr>
<tr>
<td>Sex: Male</td>
<td>19</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Tumour: Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>6</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Temporal</td>
<td>10</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Parietal</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Occipital</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Thalamic</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Brain-stem</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Grade (Astrocytomas)‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>11</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Grade 3</td>
<td>16</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Average duration (months) of clinical history</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(3 wks–3 yrs) §</td>
<td>(5 wks–5 yrs)</td>
<td>(2 wks–8 yrs)</td>
</tr>
</tbody>
</table>

*Per cent of total series.
†Range from youngest to oldest patient.
‡Kernohan et al., 1949.
§Range from shortest to longest duration.

detailed clinical data were available only in 18 cases but, with the exception of 2 cases with tumour symptoms of more than one year, all these had short clinical histories lasting only a few weeks or months (average six months). In 17 of the 18 cases a neurosurgical operation had been performed within a month of death (14 had had the tumour biopsied and in 3, part of the tumour had been removed). No case had received radiotherapy.

Group B

A slight lymphocytic reaction to the tumour was present in 26 cases (28 per cent of the series). All were adults except for one child aged 12. In this child and in 2 of the adults the glioma had arisen in the brain-stem, but the commonest tumour site in this group was the frontal lobe. Tumour malignancy based on grading was marginally less than in Group A and the duration of symptoms judged from the 22 Group B case histories available was slightly longer—eight months. Biopsy of
the tumour had been performed in 14 of the 22 cases during the month before death and 2 other cases had been given radiotherapy six and eight months respectively before death.

**Group C**

In this, the largest group of 39 cases comprising 42 per cent of the series no lymphocytes were observed in the tumours which included 2 oligodendrogliomas, 2 medulloblastomas and 3 ependymomas. One medulloblastoma had occurred in a child of 5 and another child of 4 had died of a brain-stem astrocytoma. With the exception of 2 other adults with brain-stem astrocytomas the remaining tumours were cerebral astrocytomas of adults, all but 3 of which had arisen in either the frontal or temporal lobe. The malignancy of Group C tumours was rather less than that of Groups A and B. 19 of the astrocytomas were of Grade 3 malignancy with 8 Grade 4 and 5 Grade 2 tumours. The average duration of tumour symptoms obtained from the 33 cases with adequate data was eleven months compared with six and eight months respectively in Groups A and B. 15 patients had had a biopsy and 3 partial removal of the tumour in the month preceding death. 3 other patients had had radiation therapy ten, twelve and eighteen months before death.

**Characters of the Infiltration**

The size of the sample in each instance was admittedly small for only 1–4 slides from different parts of the tumour were available. Moreover, being a retrospective study the tissue blocks had not been taken to select the different regions of the edges of each tumour, although in the great majority of sections the tumour edge was present. Furthermore, the presence of necrosis and haemorrhage within the tumour as a whole, which could conceivably affect whether infiltration occurred or not, could not be adequately assessed, although necrotic areas, often extensive, occurred in the sections of most of the tumours.

In all of the Group A cases the greater part of the small round cell infiltration consisted of perivascular cuffing of vessels in the brain parenchyma in the immediate vicinity of the tumour edge. Usually the cuffs of cells were from 2–6 cells thick and in most instances the cells were considered to be lymphocytic in nature. Plasma cells were particularly sought for, but were present in significant numbers only in 1 case in Group A and in 1 case in Group B (fig. 3). Most, but not all, of the infiltrating cells were seen near areas of the tumour that had undergone necrosis, but the significance of this correlation is uncertain, because necrosis often formed a large component of the tumour material in the slides, and the block sampling had not been designed to determine this point. None the less, in several samples infiltration was present near areas of tumour without necrosis.

In addition to perivascular cuffing, diffuse lymphocytic infiltration, either along the tumour edge or actually within the tumour itself, occurred in 3 Group A cases. However, it never reached the degree found in malignant tumours in other tissues, e.g. breast (Hamlin, 1968).
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DISCUSSION

Clearly there are many disadvantages of using such limited post-mortem material for a study of this kind, not the least being the entirely random collection of block samples from the brain tumours. None the less, it can be stated on the basis of this survey that lymphocytic infiltration may be a noteworthy feature of fatal gliomas, since 30 per cent of the tumours examined fell into Group A. The important question raised by this observation is whether the lymphocytic infiltration represents an attempt by the host to reject the living tumour or whether it is simply a consequence of surgery, irradiation or spontaneous necrosis of the tumour.

The part played by operation in producing the lymphocytic infiltration can be discounted. Although a biopsy had been performed on 17 of the 18 cases in Group A, in 8 of these the patient had died within one to seven days. The character of the infiltration was no different from that of the remaining cases which survived for several weeks after the operation. Moreover, similar operations had been carried out in Group B and C without apparently influencing this feature. The possible role of radiotherapy can also be dismissed since irradiation was performed only in 2 instances in Group B and 3 in Group C. The question as to whether the lymphocytic infiltration was a response to degenerative changes in necrotic portions of the tumour is difficult to answer. Indeed, it cannot be answered on purely histological grounds. Nevertheless, two points about the relationship of the lymphocytic infiltration and necrosis deserve mention. Necrotic changes apparently occurred within the tumour as often in cases of Group C as in Group A, and in the former no significant amount of lymphocytic infiltration was found. This fact could mean that either the host response was poor in Group C or that the degeneration products were a feeble stimulus in these cases. The finding that similar degrees of infiltration occurred in Group A both near viable tumour and near areas of necrosis suggests that the infiltration was not necessarily the result of degenerative changes produced in the tumour by other causes.

The degree of lymphocytic infiltration around gliomas appears to be less than that commonly found in cancer of the breast (Hamlin, 1968) stomach (Black, Opler and Speer, 1954, 1956) and a number of other cancers (Stewart, 1969). Only 15 per cent of cancers in Stewart's series showed no lymphocytic reaction whereas lymphocytic infiltration was absent in 42 per cent of the present gliomas, many of which were highly malignant, and in another 28 per cent the response was slight. An important point to bear in mind, however, is that the glioma material was obtained post mortem whereas in all the other studies cited, operation specimens were examined. It is now considered that large tumours, by producing excessive quantities of antigens can exhaust, or even paralyse, the host's immunological defences (Turk, 1969). Terminal glioma patients may not, therefore, be the most suitable cases to examine. Biopsy material from early cases would probably afford a better guide to the degree of cellular response that may be expected. In this respect it should be mentioned that the greatest degree of lymphocytic cuffing we have yet encountered was in a
biopsy from a Grade 3 astrocytoma in the temporal lobe of a 54-year-old man. This specimen (fig. 4) was kindly lent to us by Dr. R. O. Barnard.

There is another possible explanation for the relatively poor lymphocytic response observed in gliomas, that the surface antigenicity of glioma cells may be masked by a sialomucinous coat. Coating of trophoblastic cells by sialomucinous material is now considered to be the probable reason why no maternal immune response is mounted against paternal antigens in the intra-uterine fetus (Billington, 1969). Sialomucin by reason of its high net negative charge may also have a repelling effect on lymphoid cells. An analogous situation seems to exist in allogeneic grafts of cartilage (Heyner, 1969) which may survive without necrosis for more than a month even, under certain circumstances, in the presence of a host cellular immune response. Malignant astrocytomas are sometimes rather mucinous tumours with a slimy consistency in the fresh state and they often develop mucin containing cysts. Oligodendrogliomas are even more mucinous in character and it is noteworthy that both oligodendrogliomas of the present series fell into Group C. The presence of a coat of sialomucin around tumour cells may make a considerable difference as to whether or not an adequate immune response is produced against them. Thus Sanford (1967) has shown that previous treatment of a transplantable (TA3) tumour with neuraminidase or the administration of neuraminidase to the host after implantation markedly increased the host survival, and Currie and Bagshawe (1968) report similar experiences with the Landschütz ascites tumour. We suggest that the concealment of surface antigens in glioma cells may be an important consideration in any studies directed towards specific immunotherapy.

SUMMARY

Post-mortem material from 93 cases of glioma dying at the London Hospital between 1959 and 1966 was examined to assess the frequency and degree of lymphocytic infiltration in gliomas. Thirty per cent of gliomas showed significant lymphocytic infiltration, and a further 28 per cent showed slight infiltration. No lymphocytic reaction was present in the remaining 42 per cent.

The possibility that the lymphocytic reaction is simply a response to necrosis within the tumour cannot be excluded, but evidence is discussed which supports the idea that the response may represent an attempt by the host to reject the tumour. Factors that may govern the immunogenicity of gliomas are also considered.

ACKNOWLEDGMENTS

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LEGENDS FOR PLATES

PLATE IX

Fig. 1.—Moderate small round cell cuffing near a glioma. The brain parenchyma is lightly infiltrated with small round cells which can be seen diffusing out from the vessel in the lower part of the photograph. H. and E. ×140.

Fig. 2.—Marked small round cell cuffing of brain parenchyma near a glioma. Numerous small round cells are scattered throughout the parenchyma. H. and E. ×70.

PLATE X

Fig. 3.—Plasma cells (arrowed) amongst small round cells in a cuffed vessel near the edge of a glioma. H. and E. ×560.

Fig. 4.—Biopsy of a Grade 3 glioma. Dense small round cell perivascular cuffing at edge of tumour which extends across upper border of photograph. The brain parenchyma is diffusely infiltrated with small round cells. H. and E. ×70.

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