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Natural Killer Activity of Blood Lymphocytes in Patients with Primary Intracranial Tumors. Correlation to Histological Tumor Type and Anatomical Site

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Abstract—*The possible relationship between the natural killer (NK) activity of blood lymphocytes and the histological tumor type or anatomical location of the lesions was examined in 116 patients with primary intracranial tumors. The patients had not undergone any surgical intervention or received any treatment with ionizing radiation or cytotoxic drugs. However, some of them had received corticosteroid medication. Regardless of the histological type of tumor, there was no significant difference in the NK activity of the non-corticosteroid treated patients and the healthy control subjects. However, there was a trend towards an increased NK-activity in patients with low-grade gliomas, in particular oligodendrogliomas. The NK-activity was reduced in patients who were treated with corticosteroids. There was no relationship between the NK-activity in non-steroid treated patients and the anatomical location of the tumor. The latter finding contrasts to a recent observation showing a strong relationship between tumor site and PPD-reactivity of blood lymphocytes in patients with intracranial tumors.*

INTRODUCTION

IT HAS been reported that most patients with primary intracranial tumors have severely depressed immunological competence. For instance they exhibit a reduced capacity to develop delayed cutaneous hypersensitivity reactions to microbial antigens [1, 2] and to become sensitized to new antigens like dinitrochlorobenzene [1–4]. Since blood lymphocytes from such patients were observed to exhibit subnormal proliferative responses to polyclonal mitogens and various antigens *in vitro* [1–7] it has been suggested that at least a part of the immunological impairment is due to a defect T-cell population. This hypothesis was supported by several investigators who reported a reduced frequency of T-cells in the blood [4, 8, 9] and altered membrane characteristics of T-cells in patients with primary intracranial tumors [6, 9, 10]. The possibilty that reduced immunological reactivity could be due to inhibitory serum factors has also been proposed [1, 2, 4, 5, 7].

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Theoretically the immunological dysfunctions of patients with brain tumors may be caused by either factors which are released by the malignant cells [11, 12] or the tumor itself may, by the nature of its anatomical position, interfere with the immunoregulatory function of certain centers in the brain. In rodents, for example, such centers have been mapped to the left cerebral cortex [13–15] and parts of the hypothalamus [16–24].

In order to further understand the mechanisms responsible for the immunosuppressed state of brain tumor patients we have tested various immunological functions of blood lymphocytes obtained from more than 100 untreated patients. In a previous study we reported that there was only a weak association between histological tumor type and the capacity of the patients' blood lymphocytes to respond to phytohemaglutinin (PHA) and purified protein derivative of tuberculin (PPD) *in vitro* [25]. However, there was a strong relationship between the site of the tumor and PPD-reactivity of the blood lymphocytes [26]. In the present investigation we have examined the natural killer (NK) activity of blood lymphocytes obtained from 116 patients with untreated primary intracranial

tumors to determine if this lymphocyte function correlates to tumor type or anatomical location.

MATERIALS AND METHODS

Patients

A total number of 130 patients were examined. Some of these patients have been included in a previous study [25]. Computerized tomography of all these patients had indicated the presence of a primary intracranial tumor. At the time of blood sampling the lesions were not biopsied and the patients had not undergone any treatment with ionizing irradiation or cytotoxic drugs. However, a fraction of the patients were receiving corticosteroid medication (see below).

Specimens for histological examination were obtained from all the patients, either during open surgery or from stereotactic biopsies. A diagnosis of a primary intracranial tumor was made in 116 cases. Fourteen patients were excluded from the study. In six of them the lesions were metastatic arising from primary tumors outside the central nervous system (CNS) and the aetiology of the lesion in the other eight patients was non-malignant.

Seventy-four healthy control volunteers, who were matched for sex and age (usually ± 5 yr), were tested in parallel.

Details of patient characteristics are shown in Table 1.

Grouping of the patients according to histopathological tumor type

Histopathological classification was carried out according to the recommendations of WHO [27]. In this study the tumors have been divided into the following four main groups: (I) oligodendrogliomas, (II) astrocytomas, (III) malignant gliomas and (IV) miscellaneous tumors. Patients with oligoastrocytomas have been tabulated with both the oligodendrogliomas and the astrocytomas. The malignant glioma group was comprised of patients with anaplastic astrocytomas and glioblastomas. The tumor types included in the miscellaneous group are shown in Tables 1 and 2.

Table 1. *Characteristics of non-corticosteroid treated patients and controls. NK-activities of these patients are presented in Fig.* 1

	Males; Females	Age range and mean age (yr) Patients	Controls
Oligodendrogliomas*	5;5	31–70 (51)	26–64 (48)
Astrocytomas	13;5	20–65 (41)	21–60 (38)
Malignant gliomas	11;15	23–73 (52)	25–64 (49)
Miscellaneous tumors†	8;13	33–76 (53)	34–66 (49)

*Four of the patients had mixed tumors. One of them is also included in the astrocytoma group and three in the malignant glioma group.
†Sixteen of the patients had meningiomas, one chordoma, one hemangioblastoma, one cerebellar astrocytoma grade I, one Schwannoma and one patient had a reactive gliosis alternatively as astrocytoma.

Table 2. *Characteristics of corticosteroid treated patients and controls. NK-activities of these patients are presented in Fig.* 2

	Males; Females	Age range and mean age (yr) Patients	Controls
Oligodendrogliomas*	4;4	25–64 (40)	22–62 (38)
Astrocytomas	4;3	25–75 (42)	22–60 (36)
Malignant gliomas	17;12	15–74 (56)	22–64 (52)
Miscellaneous tumors†	3;2	22–73 (49)	21–63 (44)

*Four of these patients had mixed tumors. Three of them are also included in the astrocytoma group and one in the malignant glioma group.
†Two of these patients had meningiomas, one craniopharyngeoma, one ependymoma and one medulloblastoma.

Grouping of the patients according to tumor site

Based on roentgenological examinations performed prior to our tests the patients were divided into the following groups depending on the intracerebral (mainly gliomas) or extracerebral (mainly meningiomas) location of the tumor as described before [26]. (I) Tumors growing in the left cerebral hemisphere or outside but adjacent to this part of the brain. Tumors extending into the midline structures of the brain such as the central ganglia or *corpus callosum* have been excluded. (II) The same criteria were used for the right cerebral hemisphere. (III) Tumors involving central structures of the brain. These included structures such as *corpos callosum*, central ganglia and other parts of the brain stem down to the spinal cord. Hemispheric tumors penetrating into these structures were included in this group. (IV) Tumors confined to the posterior fossa of the skull.

Medication

Since corticosteroids may be strongly immunosuppressive the patients were divided into those who had never received such therapy and those who were either currently receiving corticosteroid treatment or had done so in the immediate past.

Separation of blood lymphocytes

Lymphoid cells were separated from heparinized venous blood as described by Bøyum [28]. Depletion of monocytes from cell suspensions was achieved using iron powder and a magnet [29].

NK-cell assay

The assay was carried out as previously described [30]. Briefly, various numbers of lymphocytes were incubated with 10^4 51-Cr-labelled allogeneic target cells termed K562 and Chang at ratios indicated in the text. The former cell line was derived from a patient with a myeloid leukemia and the latter from human liver. After 4 hr of incubation at 37° C the proportion of released isotope was measured and a cytotoxic index was calculated as follows:

$$\frac{\%\text{ release with lymphocytes} - \%\text{ spontaneous release}}{100 - \%\text{ spontaneous release}}$$

All tests were performed in duplicate.

Data processing and statistical evaluations

Mean cytotoxic indices and standard errors (M ± S.E.) were calculated on a $\log_{10}$ basis because the distributions of the determinations were skew. Since some of the values were 0 or close to 0 the lowest value was arbitrarily put as 0.01. Statistical significance between mean values of patients and healthy control volunteers tested in parallel were calculated using the paired Student's *t*-test.

RESULTS

Relation between NK-activity and histopathology of the tumors

Non-corticosteroid treated patients. A total number of 71 patients were studied. The age and sex distribution of these patients, grouped according to tumor type, and their respective controls are shown in Table 1.

Figure 1 shows that the NK-activity of the patients' lymphocytes did not differ from that of the controls. Although not statistically significant the NK-activity of lymphocytes obtained from patients with oligodendrogliomas and astrocytomas tended to be increased against Chang cells. The pooled values of these two patients groups ($n = 25$), with relatively benign gliomas, show a significantly enhanced NK-cell activity at all lymphocyte : target cell ratios tested ($P < 0.05$) (data not shown). NK-activity against the K562 cells tended to be increased in the oligodendroglioma but not in the astrocytoma group of patients.

Corticosteroid treated patients. Forty-five of the patients had received corticosteroid treatment. The age and sex distribution of these patients and their controls are shown in Table 2 and the steroid doses and duration of treatment at the time of the tests are shown in Table 3.

Figure 2 shows that the NK-activity was significantly depressed against the K562 cells in patients with oligodendrogliomas and malignant gliomas. A similar trend was observed in patients with astrocytomas and miscellaneous tumors. Statistically significant reductions of NK-activity against Chang cells were observed in oligodendrogliomas and malignant gliomas (data not shown). The patient numbers in the other two groups was too small for statistical analysis.

Relation between NK-activity and tumor site

The non-corticosteroid treated patients were divided into four groups according to their intracranial site of the tumor as described above. The results of NK activity in patients with tumors in the posterior fossa are not included because of insufficient numbers of patients.

Figure 3 shows that there were no significant differences in NK-activity against Chang or K562 cells between patients and controls regardless of anatomical site.

DISCUSSION

In a previous article we reported that PHA- and PPD-responses of blood lymphocytes in untreated

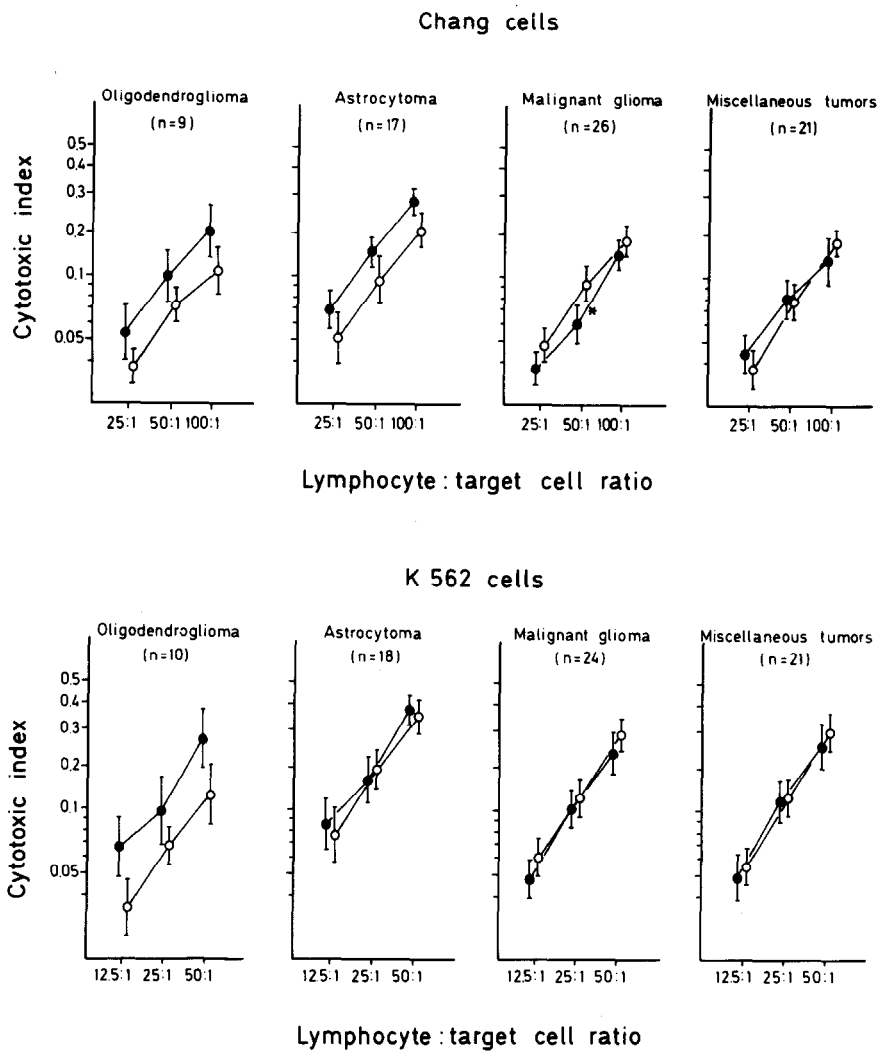

*Fig. 1. NK-activities of blood lymphocytes obtained from non-corticosteroid treated patients with various histopathological types of primary intracranial tumors. The target cells used were Chang (a) and K562 (b) M±S.E. are shown. ●——● patients, ○——○ controls. Statistical significance between patients and controls is indicated by an asterisk. *P < 0.05.*

*Table 3. Doses of corticosteroids given to the patients at the time of the tests and duration of treatment**

	Daily corticosteroid dose (mg)		Duration of therapy† (days)	
	Range	M	Range	M
Oligodendrogliomas	3–16	14.3	1–53	10.6
Astrocytomas	16	16	1–20	5.3
Malignant gliomas‡	0–16	12.9	1–110	15.5
Miscellaneous tumors	6–16	13.5	1–3	1.7

*The steroids used were given by the oral route and consisted of dexamethazone and betamethazone.

†Some of the values are approximations.

‡In one patient steroid treatment was discontinued 16 days before the test.

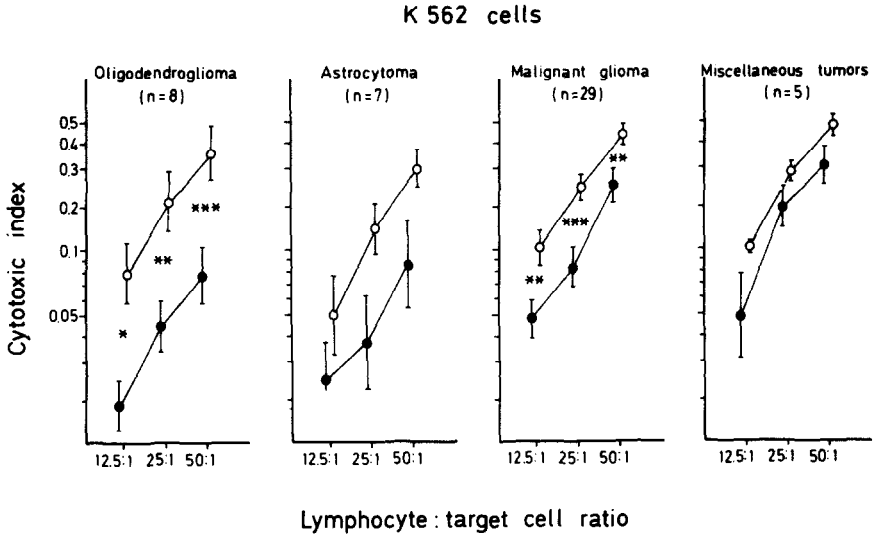

Fig. 2. NK-activities of blood lymphocytes obtained from corticosteroid treated patients. Symbols as in Fig. 1. $^{**}P < 0.01$, $^{***}P < 0.001$.

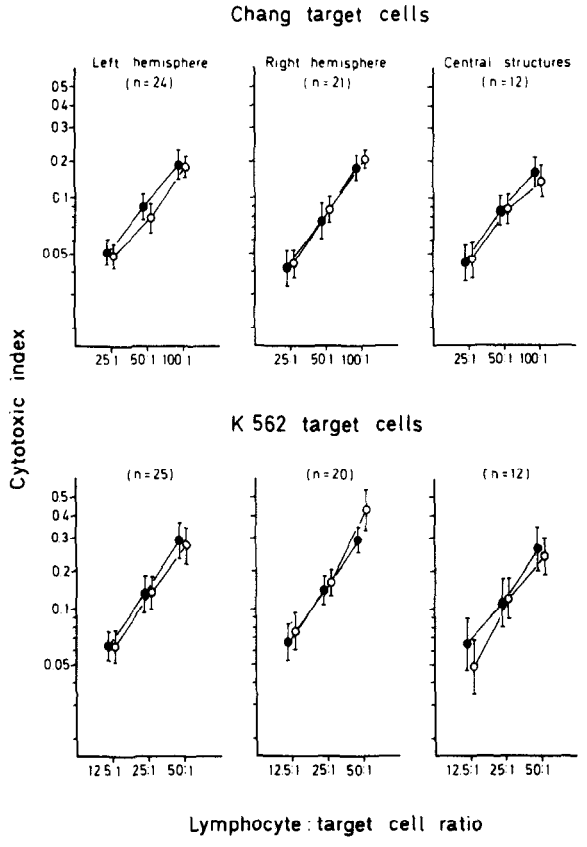

Fig. 3. NK-activities of blood lymphocytes obtained from non-corticosteroid treated patients. The patients are grouped according to the anatomical site of the tumors. Symbols as in Fig. 1.

patients with intracranial gliomas, regardless of tumor type, are essentially similar to those of sex and age matched control individuals [25]. This finding was in a sharp contrast to those of other investigators (see Introduction). However, the results did show a strong association between PPD-reactivity of the lymphocytes and the anatomical location of the tumors. As a group, patients with tumors growing in the central parts of the brain exhibited extremely low PPD-responses, but patients with tumors confined to either of the two cerebral hemispheres or the posterior fossa of the skull were normal in this respect [26]. One explanation of these previously undescribed results

could be that tumors growing in the central parts of the brain interfere with immunoregulatory function(s) of certain brain centers. This hypothesis is supported by the results of several animal experiments. These show that the electrical activity of neurons in the hypothalamus is increased during an immune response [31] and that lesions in certain parts of the hypothalmaus can reduce T-cell dependent immune responses [16–23].

In the present investigation we examined the NK-activity of blood lymphocytes in patients with primary intracranial tumors. The results showed that this lymphocyte function is normal in patients with malignant gliomas (anaplastic astrocytomas and glioblastomas) and in a group of patients with miscellaneous tumors (mainly meningiomas). However, this lymphocyte function may be enhanced in patients with oligodendrogliomas and possibly also astrocytomas (see Fig. 1). This finding is of interest since there is strong evidence that sera of patients with oligodendrogliomas contain immunomodulatory factors. It has been shown that sera from such individuals, but not from patients with other types of intracranial tumors can suppress mitogenic responses of lymphocytes [32]. In analogy with the interferons, these factors may augment NK-activity of lymphocytes [33].

The importance of taking corticosteroid medication into consideration when studying various immunological functions is emphasized by the results shown in Fig. 2.

Recently, Cross *et al.* reported that the anterior parts of the hypothalamus may regulate NK-activity in rats [24] and Bardos *et al.* showed that NK activity is to some extent under control of the left cerebral cortex [13]. These studies prompted us to examine the relationship between blood NK-activity and the anatomical location of the tumors in the non-steroid treated patients. The results presented in Fig. 3 failed to demonstrate any association between the two parameters. The findings that PHA-responsiveness [26] and NK-activity of lymphocytes in patients with centrally growing brain tumors is unchanged but PPD-reactivity is sharply reduced [26], favours the idea that centers in the mid-line structures are mainly involved in the regulation of T-cell memory.

In conclusion, the results of this investigation suggest that the NK-activity of blood lymphocytes may be enhanced in patients with low-grade intracranial gliomas, in particular oligodendrogliomas. The fact that we were unable to demonstrate a relationship between the tumor site and the blood NK-activity does not prove that such a relationship does not exist in the human since statistical analysis of the anatomical subgroups was not possible.

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