Eur J Cancer, Vol. 29A, No. 15, pp. 2118–2125, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Cytokine Gene Expression in Primary Brain Tumours, Metastases and Meningiomas Suggests Specific Transcription Patterns

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To obtain an insight into the network of cytokine gene transcription in the brain tumour microenvironment, we investigated the expression of genes encoding for interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, interferon (IFN)-γ, granulocyte–macrophage colony-stimulating factor, tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1, -β2 and -β3 in freshly excised brain tumour samples and autologous peripheral blood mononuclear cells. Tissue specimens from 15 primary brain tumours, three brain metastases, five meningiomas, autologous peripheral blood mononuclear cells (PBMC) and three brain tumour cell lines were tested by reverse polymerase chain reaction. Despite the presence of T-lymphocytes, cytokine gene transcripts typically detectable upon T cell receptor triggering could not be observed in central nervous system tumours of diverse histology. In primary brain neoplasms, transcription of genes encoding for the inhibitory cytokines TGF-β and IL-10 was detectable in more than 50% of samples. IL-6 transcripts could only be detected in malignant gliomas. In brain metastases, virtually no cytokine gene transcripts could be observed. Surprisingly, TGF-β transcripts were also detected in all meningiomas. Thus, transcription of genes encoding for inhibitory factors appears to prevail in primary brain neoplasms.
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INTRODUCTION

INFILTRATION OF neoplastic tissue by T cells and macrophages can be observed in many types of cancers [1]. Although the exact role of these cells remains controversial, their presence is often interpreted as a sign of an immune reaction against resident tumour cells [2]. Clinical extension of this concept has led to immunotherapy trials with cultured tumour-infiltrating lymphocytes (TIL) [2]. Infiltrating lymphocytes have been shown to express activation markers [3] and to display restricted usage of T cell receptor variable genes in comparison with autologous peripheral blood lymphocytes [4].

Cytokines are soluble factors controlling a wide range of leucocyte functions including activation, growth, differentiation and functional inhibition [5]. While several studies have been performed to elucidate their functions in the response to infectious challenges, their role in the tumour microenvironment *in vivo* is still not well understood.

Tumour cells can produce various cytokines, some endowed with inhibitory effects. As an example, glioblastoma cell lines have been shown to produce suppressive factors, such as members of the transforming growth factor (TGF)-β gene family and prostaglandin E2 [6, 7]. This might account for the impaired lymphocyte functions in patients with glial tumours [8]. On the other hand, tumour-infiltrating cells, if functionally competent, could also transcribe cytokine genes and secrete specific proteins, possibly upon stimulation by putative tumour antigens.

In order to obtain data closely reflecting *in vivo* cytokine gene expression, without risk of artifacts inherent to cell separation, we investigated the transcription of genes encoding for an extended panel of cytokines in freshly excised central nervous system (CNS) tumour biopsies. Reverse polymerase chain reaction (PCR), a method characterised by extreme sensitivity in detecting low amounts of mRNA, was employed.

Our data indicate that transcription of lymphokine genes, such as interleukin (IL)-2, IL-4, interferon (IFN)-γ and granulocyte–macrophase colony-stimulatory factor (GM-CSF), as observed upon antigen stimulation of T cells, is nearly undetectable in lymphocyte infiltrated CNS tumours of different histology. On the other hand, IL-1α, IL-1β, tumour necrosis factor (TNF)-α and IL-6 genes were found to be transcribed to a variable extent (20–100%) in brain neoplasms and meningiomas. Moreover, mRNA of the suppressive factors TGF-β and IL-10 could frequently be detected in primary brain tumours. Surprisingly, TGF-β transcripts were also detected in all meningiomas. Brain metastases were negative for virtually all cytokines analysed. These data suggest different patterns of cytokine gene expression in CNS tumours.

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Revised 29 June 1993; accepted 26 July 1993.

MATERIALS AND METHODS

Patients data and clinical characteristics

Table 1 summarises clinical data of patients whose tumours were studied. Fifteen primary brain tumours, three brain metastases and five meningiomas were investigated for cytokine gene transcription. Except for 2 cases (G46 glioblastoma and G49 low-

*Table 1. Patients' data and clinical management of primary malignant brain neoplasms**

Case no. (sample)	Age (years)	Sex	Diagnosis (WHO grade)	Location	Steroids	Anticonvulsants
Primary CNS neoplasms						
1 (G40)	61	M	Glioblastoma multiforme (IV)	L-O	Yes	Yes
2 (G45)	67	M	Glioblastoma multiforme (IV)	R-F-P	Yes	Yes
3 (G46)	75	F	Glioblastoma multiforme (IV)	R-F	No	Yes
4 (G51)	76	F	Glioblastoma multiforme (IV)	L-P	Yes	Yes
5 (G52)	75	F	Glioblastoma multiforme (IV)	R-T	Yes	Yes
6 (G26)	73	F	Anaplastic astrocytoma (III)	R-P	Yes	Yes
7 (G22)	57	M	Oligoastrocytoma (astrocytoma III)	R-F	Yes	Yes
8 (G50)	75	M	Anaplastic oligodendroglioma (III)	R-P	Yes	Yes
9 (G8)	13	F	Anaplastic astrocytoma (II-III)	L-T	Yes	Yes
10 (G38)	50	M	Oligoastrocytoma (astrocytoma II)	L-O	Yes	Yes
11 (G49)	56	F	Astrocytoma (II)	L-T	No	Yes
12 (G48)	17	F	Pilocytic astrocytoma (I)	L-F-T	Yes	Yes
13 (G19)	3	M	Medulloblastoma (PNET)	Vermis	Yes	No
14 (G42)	26	F	Medulloblastoma (PNET)	Vermis	Yes	No
15 (G47)	2	M	Ependymoma	4th ventricle	Yes	No
Brain metastases						
16 (G31)	53	M	Adenocarcinoma, unknown origin	L-T	Yes	Yes
17 (G36)	68	F	Rectal carcinoma	CBLL	Yes	No
18 (G41)	73	M	Adenocarcinoma of prostate	L-F	Yes	Yes
Benign tumours						
19 (G20)	42	F	Meningioma (transitional)	CL	Yes	Yes
20 (G23)	42	M	Meningioma (transitional)	Olf	Yes	Yes
21 (G24)	23	F	Meningioma (psammomatous)	Spinal	Yes	No
22 (G25)	66	F	Meningioma (transitional)	Sph	Yes	Yes
23 (G39)	50	F	Meningioma (fibroblastic)	Paras.	Yes	Yes

*Status at surgery for primary and secondary tumours of the CNS: chemo- and/or radiotherapy had not been performed prior to surgery. M, male; F, female; PNET, primitive neuroectodermal tumour; R, right; L, left; F, frontal; T, temporal; P, parietal, O, occipital; CBLL, cerebellar; CL, clivus; Olf, olfactory groove; Sph, sphenoid wing; Paras., parasagittal.

grade astrocytoma), all patients had received dexamethasone perioperatively. In addition, all but 5 patients (G19, G24, G36, G42, G47) were treated with phenylhydantoin.

Isolation of peripheral blood mononucleur cells (PBMC)

Autologous venous blood samples were obtained from the patients during surgery. PBMC were separated by gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway). Total RNA was subsequently extracted, as detailed below.

Tumour cell lines

Tumour tissue obtained from glioblastoma multiforme G45 and medulloblastoma G42 patients, were mechanically disrupted and finely minced. Cells were then cultured in six-well trays (Costar, Cambridge, Massachusetts, U.S.A.) in medium consisting of RPMI 1640 supplemented with L-glutamine (2 mmol/l), penicillin (50 U/ml), streptomycin (50 mg/ml) and 10% fetal calf serum (FCS). Cell lines obtained were characterised by commercially available antibodies directed against epitopes of glial fibrillary acidic protein, fibronectin, tenascin, vimentin and neuronal markers (data not shown). Another glioblastoma-derived cell line (G4), previously generated in our laboratory, was also characterised likewise and tested for cytokine gene transcription.

Polymerase chain reaction

Total RNA was extracted from freshly excised tumours and simultaneously sampled autologous PBMC by the guanidinium-isothiocyanate-phenol-chloroform method [9]. RNA (1 μg) was reverse transcribed by using M-MLV reverse transcriptase (200 units) in the presence of oligo thymidine (dT) and nucleoside triphosphate (dNTP) (BRL, Gaithersburg, Maryland, U.S.A.). The cDNA thus obtained were tested for the presence of defined gene sequences in PCR (GeneAmp kit, Perkin–Elmer–Cetus, Norwalk, Connecticut, U.S.A.) performed in 20 μl volumes, by using specific primer pairs. Sequences of the primers are reported in Table 2. In order to exclude false positive results due to contaminating genomic DNA, all primer pairs amplified cDNA sequences encoded for in different exons. Gene amplification was carried out by 35 cycles, each including 40-s denaturation at 94°C, 40-s annealing at 62°C and 1-min extension at 72°C. PCR products were run on 1.5% agarose gels in the presence of ethidium bromide, and photographed under ultraviolet transillumination. Molecular weight markers providing bands at 1353, 1078, 872, 603, 310, 281 and 234 base pairs (BRL) were included in all gels. The specificity of amplified cDNA sequences was validated by correspondence to the expected size of genes and by restriction mapping [10, 11].

RESULTS

Cytokine gene transcription in 15 primary brain tumours

PCR was performed to assess the expression of cytokine genes in tumour samples and in autologous PBMC (Figs 1, 2; Tables 2, 3). β actin gene transcripts were detected in all the samples under investigation. Similarly, the presence of T lymphocytes

Table 2. Nucleotide sequences of the primer pairs used in this study

Gene	Primer*	
β-Actin	Sense	TGACGGGGTCACCCACACTGTGCCCATCTA
	Antisense	CTAGAAGCATTGCGGTGGACGATGGAGGG
CD3 δ	Sense	CTGGACCTGGGAAAACGCATC
	Antisense	GTACTGAGCATCATCTCTCGATC
IL-1α	Sense	ATGGCCAAAGTTCCAGACATGTTTG
	Antisense	GGTTTTCCAGTATCTGAAAGTCAGT
IL-1β	Sense	CTTCATCTTTTGAAGAAGAACCTATCTTCTT
	Antisense	AATTTTTGGGATCTACACTCTCCAGCTGTA
IL-2	Sense	ATCTACAGGATGCAACTCCTGTCTT
	Antisense	GTCAGTGTTGAGATGATGCTTTGAC
IL-4	Sense	ATGGGTCTCACCTCCCAACTGCT
	Antisense	CGAACACTTTGAATATTTCTCTCTCAT
IL-5	Sense	GCTTCTGCATTTGAGTTTGCTAGCT
	Antisense	TGGCCGTCAATGTATTTCTTTATTAAG
IL-6	Sense	ATGAACTCCTTCTCCACAAGCGC
	Antisense	GAAGAGCCCTCAGGCTGGACTG
IL-10	Sense	AAGGCATGCACAGCTCAGCACT
	Antisense	TCCTAGAGTCTATAGAGTCGCCA
IFN-γ	Sense	ATGAAATATACAAGTTATATCTTGGCTTT
	Antisense	GATGCTCTTCGACCTCGAAACAGCAT
TNF-α	Sense	ATGAGCACTGAAAGCATGATCCGG
	Antisense	GCAATGATCCCAAAGTAGACCTGCCC
GM-CSF	Sense	ACACTGCTGAGATGAATGAAACAGTAG
	Antisense	TGGACTGGCTCCCAGCAGTCAAAGGGGATG
TGF-β1	Sense	AAGTGGATCCACGAGCCCAA
	Antisense	GCTGCACTTGCAGGAGCGCAC
TGF-β2	Sense	AAATGGATACACGAACCCAA
	Antisense	GCTGCATTTGCAAGACTTTAC
TGF-β3	Sense	AAGTGGGTCCATGAACCTAA
	Antisense	GCTACATTTACAAGACTTCAC

*Sequences for β-actin, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ, GM-CSF and CD3-δ were derived from [11] and TGF-β1, -β2 and -β3 from [10]. Sizes of amplified fragments are the following: β actin, 661 bp; IL-1α, 808 bp; IL-1β, 331 bp; IL-2, 458 bp; IL-4, 456 bp; IL-5, 291 bp; IL-6, 628 bp; IL-10, 575 bp; IFN-γ, 494 bp; TNF-α, 694 bp; GM-CSF, 286 bp; TGF-β1, 247 bp; TGF-β2, 247 bp; TGF-β3, 247 bp.

was confirmed in all samples but one (glioblastoma G45) by using primers amplifying the CD3 δ-chain gene.

With regard to cytokine gene expression in primary brain tumours, three groups could be defined, based on the relative frequency of detection of their transcripts. Cytokine genes of the first group, including IL-1α, IL-10, TGF-β1, -β2 and -β3, were found to be transcribed in a majority (> 50%) of samples. Transcription of IL-1α or TGF-β2 genes was virtually limited to the tumours (11/15), and TGF-β2 was concomitantly detectable in PBMC in 1 case only. IL-10 (eight out of 15 tumours), TGF-β1 (eight out of 15 tumours) and TGF-β3 (11 out of 15 tumours) transcripts were also concomitantly detectable in three, seven and five out of 15 PBMC samples, respectively. Occasionally, transcripts of IL-1α and TGF-β1 and -β2 could also be observed in PBMC only (1, 1 and 2 cases, respectively).

A second group of cytokine genes, including IL-1β, IL-5, IL-6 and TNF-α genes, was found to be transcribed in 20–33% of tumours. IL-6 and IL-1β were transcribed in five out of 15 tumours each, while IL-5 and TNF-α transcripts could be observed in three tumours. Regarding PBMC, IL-1β, IL-5 and IL-6 transcripts were virtually undetectable (zero to one out of 15). In contrast, TNF-α transcripts were found in seven out of 15 PBMC samples, but only in three tumours.

The third group, consisting of IL-2, IL-4, IFN-γ and GM-CSF genes, was characterised by rare or no cytokine gene transcription (0–13%) in the tumour samples. Neither IL-2 nor IFN-γ mRNA were detected in any tumour. IL-2 gene transcription could only be detected in PBMC once. In tumours, transcripts for IL-4 and GM-CSF genes were observed in 2 out of 15 and 1 out of 15 cases, respectively, while in PBMC, they were detected twice.

Cytokine gene transcription in primary brain tumour cell lines

Three long-term tumour cell lines of glial (two) and of primitive neuroectodermal origin (PNET, one), generated in our laboratory, were found to transcribe β-actin and TGF-β2 genes. In the PNET line G42, no other gene transcripts could be observed. Gene expression for IL-1α could be detected in both glioma cell lines. In addition, the G4 cell line expressed IL-6, GM-CSF and TFG-β3 genes and the G45 line expressed IL-1β gene.

Cytokine gene transcription in brain metastases

In three different metastatic brain cancers, no cytokine transcripts could be identified, with the only exception of GM-CSF gene in a prostate carcinoma metastasis and in one PBMC sample (G31).

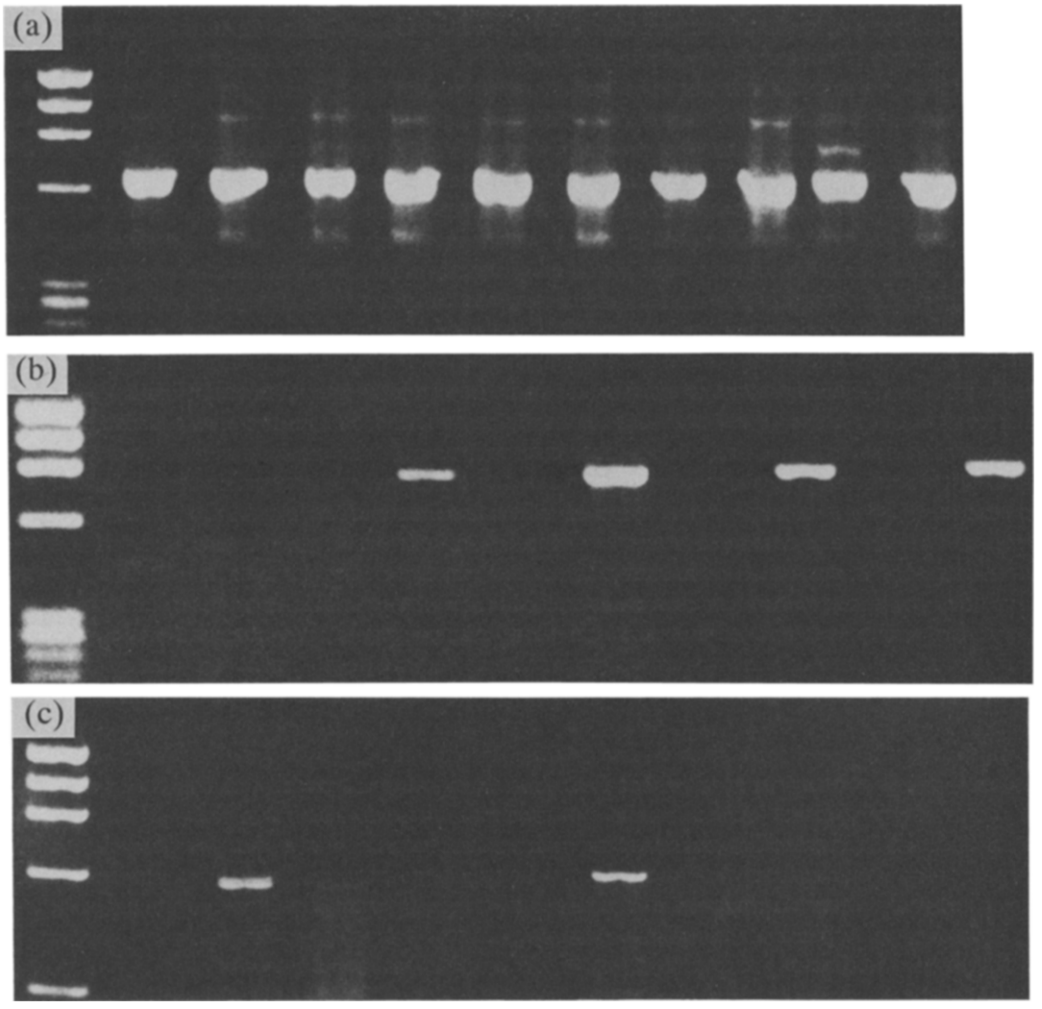

Fig. 1. Transcription of IL-1α and IL-10 genes in primary brain tumours and autologous PBMC. RNA was extracted from freshly excised primary CNS tumours G8, G19, G22, G26 and G38 (lanes 3, 5, 7, 9, 11) and from simultaneously sampled autologous PBMC (lanes 2, 4, 6, 8, 10), reverse transcribed and assayed in 35 cycles PCR for transcription of β-actin (panel a), IL-1α (panel b) and IL-10 (panel c) genes. PCR products were then run on 1.5% agarose gels in the presence of ethidium bromide, and photographed under ultraviolet transillumination. Molecular weight markers were run on lane 1. Sizes of amplified genes are reported in Table 2.

Cytokine gene transcription in meningiomas

In the five benign tumour samples, located beyond the blood–brain barrier, IL-1α, TGF-β2 and TGF-β3 transcripts could be detected in all cases. Two of the respective PBMC samples showed evidence of TGF-β3 transcription. IL-1β, TNF-α and TGF-β1 genes were found to be transcribed in three out of five tumour samples, but only TFG-β1 mRNA could also be detected in two PBMC. IL-5, IL-6 and IL-10 genes were all found transcribed in G39 tumour while GM-CSF mRNA was confined to sample G20.

DISCUSSION

Brain tumours are known to be infiltrated by monocytes and lymphocytes [12]. The functional role of these cells in the context of the interaction between the immune system and neoplasms is presently unclear. Regarding glial neoplasms, tumour infiltrating lymphocytes are characterised by severely impaired clonogenic proliferative and cytotoxic potential [4, 13, 14]. It has been hypothesised that the presence of TIL, often expressing typical 'memory cell' surface markers, could be the consequence of immune recognition of poorly defined tumour-specific antigens [15].

The generation of an efficient immune response is critically dependent on the timely and balanced secretion of a number of soluble factors, cytokines, characterised by both stimulatory and inhibitory effects on diverse cell types. Cytokines can be produced not only by immunocompetent cells, but also by other cell types, including tumour cells [5, 10]. With regard to brain neoplasms, IL-6 and TNF-α have been detected immunohistochemically in tumour cells and tumour infiltrating macrophages [16]. Depressed immune responsiveness in glioblastoma patients has been related to the production of TGF-β2 by transformed cells [17]. On the other hand, neoplastic cell lines transfected with IL-2, IL-4, IFN-γ or other cytokine genes have been shown to induce vigorous and long-lasting anti-tumour immunity [18]. Thus, local production of cytokines in the tumour mass might have profound effects on immune responsiveness, irrespective of the cell types responsible for their secretion.

Production of cytokines is mostly controlled at the post-transcriptional level. Furthermore, at least for TGF-β, the active form of the protein requires enzymatic cleavage [19]. In addition, biological effects of cytokines are dependent on interaction with specific membrane receptors or, alternatively, they can be modulated by soluble receptors. However, cytokine gene transcription represents an obvious initial step which is necessary for the production of active soluble factors.

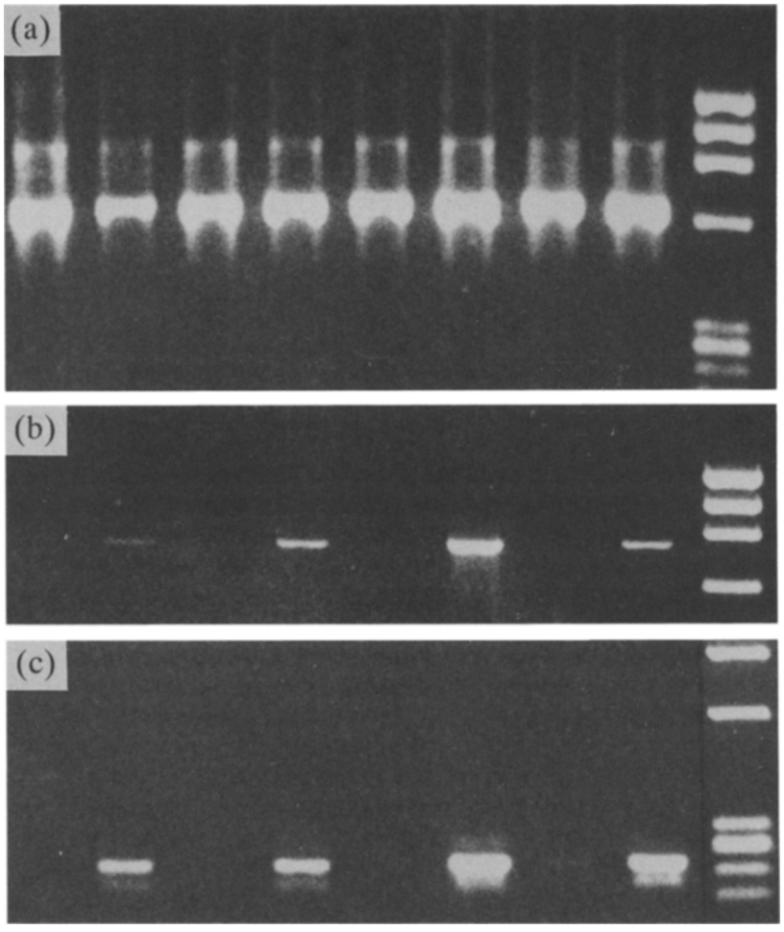

Fig. 2. Transcription of IL-1α and TGF-β2 genes in meningiomas. RNA was extracted from freshly excised meningiomas G20, G24, G23 and G25 (lanes 2, 4, 6, 8) and from simultaneously sampled autologous PBMC (lanes 1, 3, 5, 7), reverse transcribed and assayed in 35 cycles PCR for transcription of β-actin (panel a), IL-1α (panel b) and TGF-β2 (panel c) genes. PCR products were then run on agarose gels in the presence of ethidium bromide, and photographed under ultraviolet transillumination. Molecular weight markers were run on lane 9. Sizes of the amplified genes are reported in Table 2.

In this work, we investigated the expression of cytokine genes in brain tumour samples. Our approach studied cytokine gene transcription in freshly excised tumour specimens, closely reflecting *in vivo* expression within the neoplastic mass. We employed reverse PCR, which is characterised by a much higher sensitivity than northern blotting or *in situ* hybridisation techniques.

Since systemic immunosuppression has been described in CNS tumour patients [8], we also analysed cytokine gene expression in autologous PBMC. These data allowed us to clarify whether discrete cytokine transcripts, as detectable in tumour mass, were likely to be ascribed to PBMC 'trapped' in the tumour specimens. To define patterns of cytokine gene expression, possibly related to tumour histology or location on either side of the blood–brain barrier, metastatic and benign meningeal neoplasms were also studied.

Our data suggest preferential patterns of cytokine gene transcription in CNS tumour specimens. In agreement with previous reports [6, 17, 19], we found that in primary CNS tumours IL-1α and TGF-β1, -β2 and -β3 are transcribed in over 50% of cases. In addition, we observed that IL-10, a cytokine secretion inhibitory factor, is also transcribed in a majority of these neoplasms. Thus, four of the five cytokine genes most frequently transcribed in primary CNS tumours encode for well characterised inhibitors of immune responses. Transcription of IL-1β and IL-6 was detectable in a subgroup of patients, confirming previously reported data [20]. It is noteworthy that IL-6 transcripts could only be detected in malignant gliomas, thus suggesting a correlation with a defined histological tumour type. TNF-α and IL-5 genes were also found to be transcribed in three out of 15 biopsies. In contrast, transcription of IL-2, IL-4, IFN-γ and GM-CSF genes, which is typically observed upon T cell activation, could rarely or never be detected in primary CNS tumours, despite the high sensitivity of the technique employed and the detection of CD3-δ transcripts.

This array of cytokine gene transcripts could be related to steroid or anticonvulsant treatment [21, 22]. However, untreated patients do not present different cytokine gene transcripts as compared with treated. Second, transcription of genes, such as IL-1α, which could be affected by steroids [23] were predominantly detectable in tumour samples. Similarly, anaesthesia protocols do not seem to be involved, since different patterns were detectable in patients affected by different types of tumours, but undergoing similar anaesthetic procedures [24].

Taken together, our data document prevailing transcription of inhibitory cytokine genes in primary brain tumours. Further, they do not support the possibility of an active T-cell response against specific tumour antigens, since gene transcripts typically detectable upon antigen receptor triggering of T lymphocytes are virtually absent in primary brain tumours. Cytokine gene transcription in patients' PBMC raises several points of interest. Transcripts encoding for TGF-β1 were mostly concomitantly

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*Table 3. Cytokine gene expression in freshly excised primary human brain tumours and cell lines, brain metastases, meningiomas and autologous PBMC by polymerase chain reaction**

Sample	Histology	CD3	IL-1α	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	TNF-α	GM-CSF	TGF-β1	TGF-β2	TGF-β3
1(G40)	GBM	+(+)	+(−)	−(−)	−(+)	−(−)	−(−)	+(−)	+(+)	+(+)	−(−)	+(+)	−(−)	+(−)
2(G45)	GBM	−(+)	−(+)	−(+)	−(−)	−(−)	−(−)	−(−)	−(−)	−(+)	−(−)	−(+)	−(+)	−(−)
3(G46)†	GBM	+(+)	+(−)	+(−)	(−)	−(−)	−(−)	−(−)	+(+)	+(+)	−(−)	+(+)	+(−)	+(+)
4(G51)	GBM	+(+)	+(−)	+(−)	−(−)	−(−)	−(−)	+(−)	+(−)	−(−)	+(−)	−(−)	+(−)	+(−)
5(G52)	GBM	+(+)	+(−)	+(−)	−(−)	−(−)	+(−)	+(−)	+(+)	−(+)	−(−)	−(−)	−(+)	−(−)
6(G26)	AC III	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	−(−)	−(−)	+(+)	+(−)	+(−)
7(G22)	OAC III	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	+(−)	+(−)	−(+)	−(−)	+(+)	+(−)	+(+)
8(G50)	ODG III	+(+)	−(−)	−(−)	−(−)	+(−)	−(−)	−(−)	−(−)	−(−)	−(+)	−(−)	+(−)	+(−)
9(G8)	AC II-III	+(+)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	−(−)	+(+)	+(−)	+(+)
10(G38)	OAC II	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	+(−)
11(H49)†	AC II	+(+)	+(−)	+(−)	−(−)	−(+)	+(+)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)
12(G48)	AC I	+(+)	+(−)	+(−)	−(−)	+(+)	+(−)	−(−)	+(−)	+(+)	−(−)	−(−)	+(+)	−(−)
13(G19)	PNET	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(+)	−(+)	+(+)	+(−)	+(+)
14(G42)	PNET	+(+)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(+)	+(−)	+(+)
15(G47)	EPM	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	−(−)	−(−)	+(−)	+(−)
Cell lines														
A(G4)	GBM	−	+	−	−	−	−	+	−	−	+	−	+	+
B(G45)	GBM	−	+	+	−	−	−	−	−	−	−	−	+	−
C(G42)	PNET	−	−	−	−	−	−	−	−	−	−	−	+	−
Brain metastases														
16(G31)	Adeno	+(+)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	(−)	−(−)	−(+)	(−)	−(−)	−(−)
17(G36)	Rectal	+	−	−	−	−	−	−	−	−	−	−	−	−
18(G41)	Prostate	−(+)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	−(−)	−(−)
Benign tumours														
19(G20)	Mening	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(+)	−(−)	+(−)	+(−)
20(G23)	Mening	+(+)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	+(−)	+(−)	+(+)
21(G24)	Mening	−(+)	+(−)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(+)	+(−)	+(−)
22(G25)	Mening	+(+)	+(−)	+(−)	−(−)	−(−)	−(−)	−(−)	−(−)	+(−)	−(−)	+(+)	+(−)	+(+)
23(G39)	Mening	+(+)	+(−)	+(−)	−(−)	−(−)	+(−)	+(−)	+(−)	+(−)	−(−)	−(−)	+(−)	+(−)

*Total RNA was extracted immediately after tumour excision by the guanidinium-isothiocyanate-phenol method. RNA (1 μg) was reverse transcribed, and the cDNA obtained was tested for the presence of defined gene sequences in PCR reactions by using specific primer's pair. Gene amplification was carried out by 35 cycles. After PCR, the products obtained were run on 1.5% agarose gels in the presence of ethidium bromide, and photographed under ultraviolet transillumination. GBM, glioblastoma multiforme; AC, astrocytoma; OAC, oligoastrocytoma; ODG, oligodendroglioma; PNET, primitive neuroectodermal tumour, here synonymous with medulloblastoma; EPM, ependymoma. WHO grading system I–IV. † Denotes absence of perioperative dexamethasone administration. Transcripts of the β-actin gene were detectable in all samples while IFN-γ gene transcripts were always undetectable. Data in parentheses refer to autologous PBMC.

detectable in tumour and autologous PBMC. This is compatible with the possibility that peripheral blood 'contamination' could account for tumour specimen data, regarding this gene. On the other hand, IL-10, TGF-β2 and -β3 transcripts were mostly confined to tumour samples. Their concomitant detection in peripheral blood (3 cases for IL-10, 1 for TGF-β2 and 5 for TGF-β3) raises the question of their cellular origin, possibly in the context of presently undefined neuroimmune interactions [5]. Moreover, it is of relevance that IL-10 gene transcription in peripheral blood is uniquely limited to primary brain tumours, but is undetectable in metastatic tumours or in meningiomas. Conversely, the TNF-α gene is transcribed in a limited number of tumours (three out of 15), in autologous PBMC, as well as in PBMC from patients where no TNF-α transcripts could be detected in tumours (4 cases).

Metastatic and benign tumours might suggest different patterns of cytokine gene transcription. In the former, virtually no transcripts could be detected. In meningiomas, the pattern of cytokine gene expression resembled the one detectable in primary CNS tumours, as regarding IL-1α and -β, and TGF-β1, -β2, and -β3, thus in agreement with a recent report [25]. However, IL-10 transcription was only detectable in one out of five tumours. It is also of interest that IL-10 transcripts were not detectable in peripheral blood. Thus, IL-10 gene transcription might represent a relevant discriminating factor between primary CNS tumours and meningiomas both at local and systemic levels.

The data reported in this work help define patterns of cytokine genes which are transcribed in the tumour microenvironment, where neoplastic cells and immunocompetent cells interact. In primary brain tumours, transcription of cytokine genes, typically detectable in activated cells of macrophage and astrocytic lineage (IL-1α, IL-10, TFG-β) [17], can be observed. In meningiomas, these genes are also predominantly transcribed, with the relevant exception of IL-10. These transcripts are absent in the metastatic tumours tested.

With regard to IL-1α, it has been suggested that this cytokine might promote recruitment of leucocytes across the blood–brain barrier, thus playing a role in tumour infiltration of immunocompetent cells [5].

IL-10, as recently demonstrated, inhibits cytokine production and antigen-specific proliferation of T helper cell subsets [26]. Moreover, it impairs antigen presentation by downregulating expression of MHC class II molecules in macrophages and monocytes [26]. In addition, the IL-10 gene has been found to be predominantly transcribed in ovarian cancer [27] or in renal cell carcinoma biopsies [28]. Similarly, IL-10 gene mRNA has also been detected in basal cell carcinoma biopsies, where a pattern of cytokine gene transcription, reminiscent of T-helper 2 type responsiveness, has been observed [29].

In diverse pathological states of the CNS, TGF-β can be produced by cells of the monocyte–macrophage–microglia lineage, and by resident activated astrocytes [5]. In the normal adult brain, TGF-β mRNA and TGF-β proteins are not detectable [17]. There is increasing evidence that TGF-β1 and -β2 suppress the immune response by inhibiting the expression of MHC class II antigens, the growth of lymphocytes and the production of antibodies *in vivo* [30].

Data reported in this work suggest two main conclusions. First, no clear evidence for active antigen-specific T-cell responses is detectable in brain tumours. Second, transcription of genes encoding for well defined 'suppressor' cytokines, is a frequent finding in brain tumours. These conclusions are relevant to the debated issue of antigenicity of human tumours.

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Acknowledgements——This work was supported by grants from the Swiss Cancer League, Regional Cancer Leagues of Basel-Stadt and Basel-Land, and of St. Gallen-Appenzell, by San Salvatore (Lugano) and Roche Research Foundations (Basel), by Hoffmann-La Roche Ltd. (Basel), all in Switzerland, and by the Karl Mayer Foundation (Fürstentum Liechtenstein).

Thanks are due to Dr Dominique Langui from the Institute of Neuropathology, University of Basel, for immunohistochemical characterisation of tumour cell lines.

Eur J Cancer, Vol. 29A, No. 15, pp. 2125–2131, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

The Effect of Endocrine Therapy on Fibroblast Growth Factor-like Activity in Nitrosomethylurea-induced Rat Mammary Tumours

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Tumour regression following ovariectomy of rats bearing nitrosomethylurea-induced mammary tumours has been well characterised as a model for oestrogen receptor (ER)-positive breast cancer. We have shown that a similar regression response can be induced in these rats by the cytotoxic drug doxorubicin. Conditioned medium (CM) from serum-free explant cultures of the mammary tumours of ovariectomised rats showed a striking increase in its ability to transform NR6 cells compared to that of control or doxorubicin-treated rats ($P = 0.001$, t-test). Activity was also present in CM derived from rat uteri but not in ER-negative tissues such as skin and liver. Activity was further defined as fibroblast growth factor (FGF)-like by its strong affinity to heparin, partial neutralisation by antibodies to acidic FGF (aFGF) and partial co-elution with aFGF on salt elution from heparin. Both aFGF protein and mRNA were detected in tissue preparations of rat tumours and uterus.
Eur J Cancer, Vol. 29A, No. 15, pp. 2125–2131, 1993.

INTRODUCTION

ENDOCRINE MANIPULATION has long been successfully used as a treatment for human breast cancer [1]. The rat nitrosomethylurea (NMU)-induced mammary carcinoma has been extensively used as a model for anti-oestrogen-responsive breast carcinoma, and both ovariectomy and a variety of clinically useful anti-oestrogenic drugs have been shown to induce regression of these tumours [2,3]. Inhibition of oestrogen synthesis is now still the treatment of choice for the majority of breast cancer patients and many of those with metastatic disease [4].

Unfortunately, in the majority of patients endocrine therapy ultimately becomes ineffective and the cancer regrows [5]. This may be due to receptor changes or interaction with growth factors and other factors, rendering cells resistant to endocrine therapy. Cytotoxics such as doxorubicin are often also used in the treatment of breast cancer [6] and also induce tumour regression in the rat Walker mammary model [7].

The fibroblast growth factor (FGF) (of which to date there are seven members), has attracted attention in many disparate fields. Both basic (bFGF) [8] and acidic FGF (aFGF) [9] have been shown to have a wide variety of potent angiogenic effects and they may also be involved in basement membrane breakdown [10]. Both are unusual in that they code no classical signal peptide sequence, leading to speculation of a new secretion mechanism. Indeed, the main mechanism of action may be via sequestration from a basement membrane reservoir or on cell death and rupture, this latter mechanism being an obvious mechanism whereby the wound healing effects of the FGFs could be explained.

Transforming growth factors (TGF; i.e. those growth factors able to induce anchorage-independent growth of certain cell

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Revised and accepted 9 July 1993.