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EFFECTS OF INTERLEUKIN-1 AND DEXAMETHASONE ON INTERLEUKIN-6 PRODUCTION AND GROWTH IN HUMAN MENINGIOMAS

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SUMMARY

Interleukin-6 (IL-6) has been shown to be released by cultured human meningioma cells and may be a positive or negative regulator of tumour growth. IL-6 protein and mRNA levels have been examined in a series of meningiomas. In 14 cases, the results are compared with the effects of IL-6 and dexamethasone on growth and IL-6 secretion *in vitro*. Tumours with the highest *in vivo* IL-6 mRNA expression also showed maximum induction of IL-6 and increased cellular proliferation on IL-1 stimulation *in vitro*. Dexamethasone decreased the IL-1-stimulated IL-6 release in all cases. Meningiomas which had little or no IL-6 message were refractory to IL-1 control of IL-6. Remarkably, these formed the group of meningiomas that increased their growth rate in response to dexamethasone. © **1997 John Wiley & Sons, Ltd.**

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KEY WORDS—**meningiomas; interleukin-1; interleukin-6; glucocorticoids; dexamethasone**

INTRODUCTION

Meningiomas are benign tumours arising from the arachnoid layer of the meninges, are twice as common in women than in men, and account for approximately 20 per cent of all intracranial tumours.[1] Treatment is by surgical resection, but some tumours are inoperable and the recurrence rate after surgery can be high depending on the intracranial site;[2] there is no routinely available medical therapy. Results from one small study suggest that mifepristone, a glucocorticoid and progesterone receptor antagonist, may either shrink or impede tumour growth in some patients.[3] Tumour growth is enhanced during pregnancy, suggesting a possible role of the female sex steroids in their pathogenesis.[4] Progesterone and glucocorticoid receptors are present in the majority of meningiomas,[5–9] but the presence of oestrogen receptors is controversial.[6–8,10] Dexamethasone is sometimes used in patients with meningiomas to reduce oedema surrounding the tumour. The mifepristone study raises some concern as to whether or not dexamethasone may potentiate tumour growth.

The stimulation of meningioma cell growth is complex and not well understood. Epidermal growth factor (EGF) and fibroblastic growth factor (FGF) are known to stimulate growth of meningioma cells in culture,[11,12] whereas transforming growth factor-β (TGFβ) has an inhibitory effect on EGF-stimulated cell proliferation,[13] and interferon-α also inhibits growth.[14] Receptors for EGF, TGFβ, and platelet-derived growth factor (PDGF) have been identified.[13,15–17]

The cytokine interleukin-6 (IL-6) is synthesized and secreted by meningioma cells.[18,19] Expression of mRNA of other cytokines, IL-1β, IL-3, IL-8, TNFβ (tumour necrosis factor-β) and TGFβ1, β2, and β3 have been identified in meningioma tissue,[20] but apart from IL-6 and TGF, no effect on growth has been demonstrated. Reports on the effects of IL-6 on the growth of meningioma cells are diverse, with Boyle-Walsh *et al.*[18] demonstrating a stimulation of growth in 60 per cent of tumours, whereas Todo *et al.*[19] found that IL-6 inhibited proliferation. The effect of IL-6 on meningioma cell growth is clearly complex; in the first study IL-6 stimulated growth, whereas IL-1β and IL-4, although increasing IL-6 production, resulted in an overall inhibition of growth.[18] The reasons for these diverse observations are not apparent, but it has been suggested that they may be due to a concentration-dependent effect of IL-6, with inhibition at low and stimulation at high concentrations.[20] Diverse effects of IL-6 on cell growth have also been found to occur in melanomas[21] and between normal and adenomatous pituitary tissue.[22]

Expression of the IL-6 gene is controlled at its promoter by two inhibitory glucocorticoid responsive elements, a stimulatory multiresponse element (activated by IL-1, TNF, γ-interferon, bacterial lipopolysaccharide, phorbol esters, and serum) and a stimulatory cyclic AMP response element.[23] The effect of dexamethasone on cytokine actions in meningiomas has not been previously studied. We have therefore investigated the effects of dexamethasone on the growth of cultured human meningioma cells and its effect on IL-6

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Table I—Clinical data, histological classification, and comparison of IL-6 mRNA and protein levels in meningiomas*

Patient No.	Age (years)	Sex	Histology	IL-6 mRNA	IL-6 protein
1	46	F	Transitional	1	0
2	72	M	Transitional	1	ND
3	70	F	Meningoepitheliomatous	2	2
4	74	F	Meningoepitheliomatous	1	0
5	29	F	Angiomatous	0	0
6	76	M	Fibroblastic	0	0
7	46	F	Transitional	3	2
8	67	F	Meningoepitheliomatous	2	2
9	70	M	Transitional	1	ND
10	56	F	Meningoepitheliomatous	1	2
11	63	M	Transitional	4	3
12	77	M	Transitional	1	0
13	32	M	Transitional	ND	ND
14	34	M	Meningoepitheliomatous	4	3
15	62	M	Chordoid	2	0
16	68	F	Meningoepitheliomatous	0	0
17	75	F	Transitional	3	0
18	53	F	Transitional	4	3
19	79	F	Fibroblastic	1	2
20	52	M	Transitional	2	2
21	48	F	Psammomatous	ND	ND
22	76	M	Transitional	2	0
23	59	F	Fibroblastic	0	0
24	70	F	Psammomatous	1	1
25	39	F	Meningoepitheliomatous	0	0

*Scores refer to levels of staining as follows: 0 = no cells; 1 = occasional positive cells <10 per cent; 2 = 10–50 per cent positive; 3 = 50–90 per cent positive; 4 = >90 per cent positive. ND = not done.

production. The effect of IL-1 on meningioma cell growth and IL-6 secretion was studied. We have also examined the effects of exogenous IL-6 on [^{3}H]thymidine incorporation in this group of meningiomas in an attempt to clarify its effect in these tumours.

MATERIALS AND METHODS

In situ *hybridization for IL-6*

After deparaffinization and rehydration, sections from 25 meningiomas were digested at 4°C with proteinase K at a concentration of 0·01 mg/ml in phosphate-buffered saline (PBS) for 20 min, followed by post-fixation in 0·4 per cent paraformaldehyde in PBS, also at 4°C. Sections were then incubated in prehybridization buffer for 60 min at 37°C. The IL-6 antisense probe used was a cocktail of oligonucleotides labelled at both 3′ and 5′ ends with digoxigenin (R&D Systems Europe, Abingdon, Oxon, U.K.). Slides were hybridized to the probe at a concentration of 900 ng/ml overnight at 37°C followed by washing in 4 × SSC at 37°C. After preincubation with 20 per cent normal sheep serum in PBS, specifically bound probe was detected using a monoclonal antidigoxigenin antibody (Boehringer Mannheim, Lewes, East Sussex, U.K.) followed by a three-stage avidin–biotin alkaline phosphatase reaction (ABC kit; Vector Labs Ltd., Peterborough, U.K.) with nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as chromogen. An inflamed appendix was used as a positive control; no probe for a negative control; and normal grey and white matter was also tested.

Immunohistochemistry for IL-6

Sections 5 μm in thickness were cut and mounted on APES-coated slides. After dewaxing, endogenous peroxidase was blocked by immersion in 2 per cent

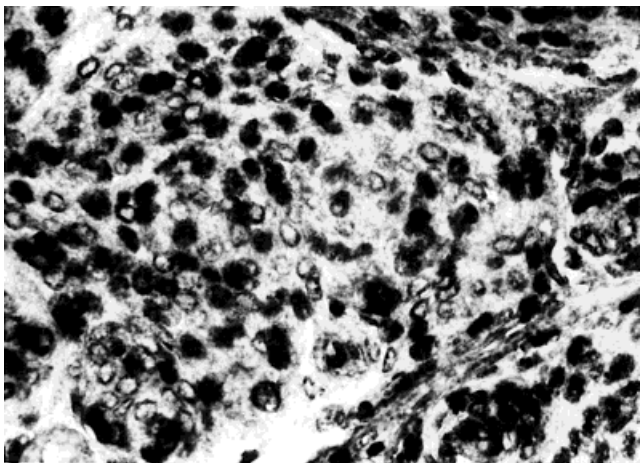

Fig. 1—*In situ* hybridization for IL-6 mRNA meningioma showing strong expression of IL-6 mRNA in most tumour cells. × 400

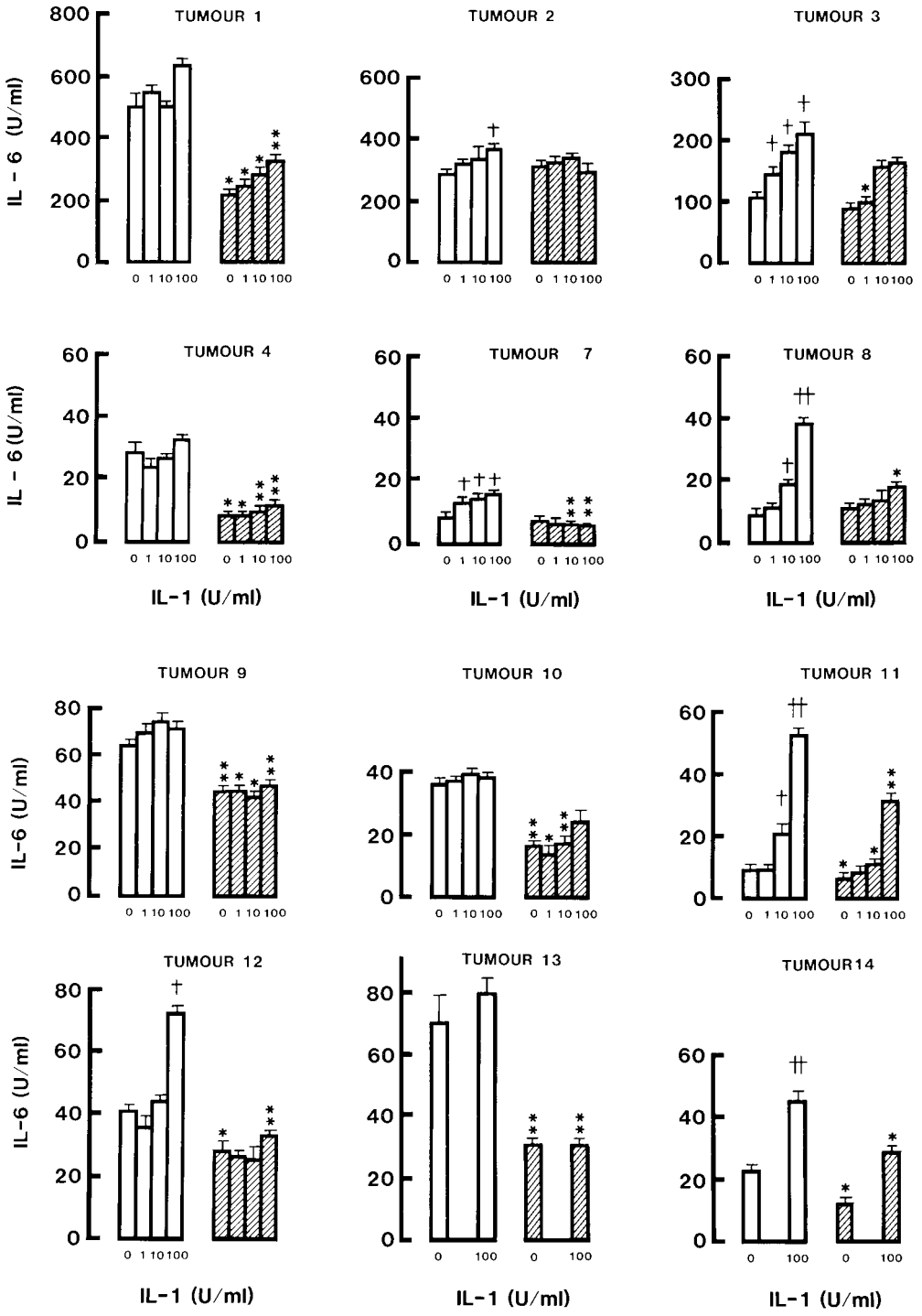

Fig. 2—Effect of dexamethasone on basal and IL-1-stimulated IL-6 production in cultured human meningioma cells. The open bars indicate cells treated only with IL-1, and the hatched bars cells treated with IL-1 in the presence of dexamethasone (100 nmol/l). The results are the mean ± SEM for triplicate wells. †$P<0{\cdot}05$, ‡$P<0{\cdot}005$ for IL-1 stimulation of IL-6 release; *$P<0{\cdot}05$, **$P<0{\cdot}005$ for dexamethasone inhibitory effect on IL-1 stimulation of IL-6 secretion

hydrogen peroxide in methanol for 10 min. Sections were then rinsed, followed by blocking in 10 per cent normal goat serum in PBS. Mouse monoclonal anti-human IL-6 (1618-01; Genzyme Diagnostics, West Malling, Kent, U.K.) was applied at a 1:200 dilution in PBS overnight at 4°C. After washing, bound antibody was detected using the ABC peroxidase system (Vector Labs) and DAB chromogen.

Cell dispersion and culture

Human meningioma tissue was obtained at surgery and transported to the laboratory in Dulbecco's

Table II—Comparison of IL-6 mRNA expression, basal IL-6 secretion with IL-1 stimulation of IL-6 release, and effects of IL-1, IL-6, and dexamethasone on [^{3}H]thymidine uptake. IL-6 mRNA expression is scored as stated in Table I

Men No.	IL-6 mRNA	IL-6 (U/ml)	IL-1 stim. IL-6	IL-1 stim. growth	IL-6 stim. growth	Dexameth stim. growth
1	1	500	0	Y	Y	N
2	1	300	1	–	–	–
3	2	100	2	N	Y	N
4	1	30	0	N	N	Y
5	0	0	0	*	*	*
6	0	0	0	*	*	*
7	3	10	2	N	Y	N
8	2	10	4	Y	–	N
9	1	63	0	N	Y	N
10	1	38	0	N inhib.	N	Y
11	4	10	5	Y	N	N
12	1	40	1	–	–	–
13	–	70	0	–	Y	–
14	4	22	3	–	N	–

N = none; Y = yes; inhib. = growth-inhibited; – = not done, because of yeast infection or insufficient cells.
*Insufficient [^{3}H]thymidine uptake.

modified Earles' medium (DMEM) with HEPES (Gibco, Paisley, U.K.). Clinical and pathological data on each of the meningiomas are presented in Table I. The tumour was trimmed of fat and fibrous tissue and washed to remove blood. It was then minced finely, suspended in PBS (pH 7·4), and filtered through sterile gauze. The tissue was resuspended in PBS with 0·5 per cent dispase (Boehringer Mannheim) and incubated at 37°C for 30 min, stirring intermittently. Again, the tissue was filtered through gauze and the supernatant was mixed with an equal volume of Medium 199 with 10 per cent fetal calf serum (FCS) and spun for 5 min at 100 rpm. The process with dispase was repeated again and the cells were plated out at a concentration of 10^5 cells per ml in Medium 199 (Flow Labs, Ayrshire, U.K.) containing 10 per cent FCS, penicillin (100 U/ml), streptomycin (100 U/ml), and fungizone (1 ml/well) in 24-well plates.

Experiments

For experiments measuring IL-6 production, the medium was removed after 4 days and test substances in Medium 199 and 10 per cent FCS were added to triplicate wells. The conditioned medium was removed after 72 h and stored at −20°C until assayed. Growth was assessed using [^{3}H]thymidine incorporation. Growth experiments were carried out on first and second passages. The cells were incubated with test substances in Medium 199 and 2 per cent FCS for 72 h with [^{3}H]thymidine added (Amersham International Plc., Bucks., U.K.), 1 μCi/well, for the last 24 h. The medium was removed and the cells were rinsed with PBS. Cell proliferation was stopped by adding 10 per cent trichloroacetic acid and the cells were solubilized overnight with 250 μl of 1 M NaOH added to each well. A 50 μl aliquot of the supernatant was added to 2 ml of scintillant (Ultima Gold XR, Packard, Groningen, The Netherlands) and counted on a scintillation counter.

Interleukin-6 assay

IL-6 was measured using an enzyme-linked immunoabsorbent assay. Flexible 96-well Costar plates (High Wycombe, Bucks., U.K.) were coated with 50 μl of polyclonal anti-IL-6 (Central Laboratory of The Netherlands, Red Cross, Amsterdam, The Netherlands) at a concentration of 2 μg/ml in a 0·55 mmol/l solution of carbonate buffer (pH 9·6) and incubated at 37°C for 2 h. Non-specific binding sites were blocked with 5 per cent BSA in Tris-buffered saline (TBS: 150 μl) overnight. The plates were washed three times in TBS containing 0·2 per cent Tween 20 between each of the following steps. Conditioned media and standards were diluted in Medium 199 and added to appropriate wells for 2 h at 37°C. After washing, 50 μl of monoclonal anti-human IL-6 (2·3 μg/ml in TBS; Central Laboratory of The Netherlands) was added to the wells for 1 h at 37°C. The plates were washed again and 50 μl of biotinylated anti-mouse IgG (1 in 2000; Amersham) was added. Streptavidin-conjugated alkaline phosphatase (Amersham) was then added in 1 in 1000 TBS with 1 per cent FCS for 30 min. Alkaline buffer solution (50 μl), 2-amino-2-methyl-1-propranolol 1·5 mmol/l, pH 10·3 (Sigma Chemical Co. Ltd., Poole, U.K.), was added to each well followed by 50 μl of phosphatase substrate (Sigma) prepared at 10 g/l in distilled water. The plates were incubated at 37°C until the colour developed and the reaction was then stopped with NaOH (50 μl, 0·1 mol/l). The absorbance was read on a Dynatech MR5000 reader at 414 nm before and after decolourization with 50 μl of 4 μmol/l HCl and the absorbance read again. The decolourized absorbance was subtracted from the initial reading to give the absorbance due to the

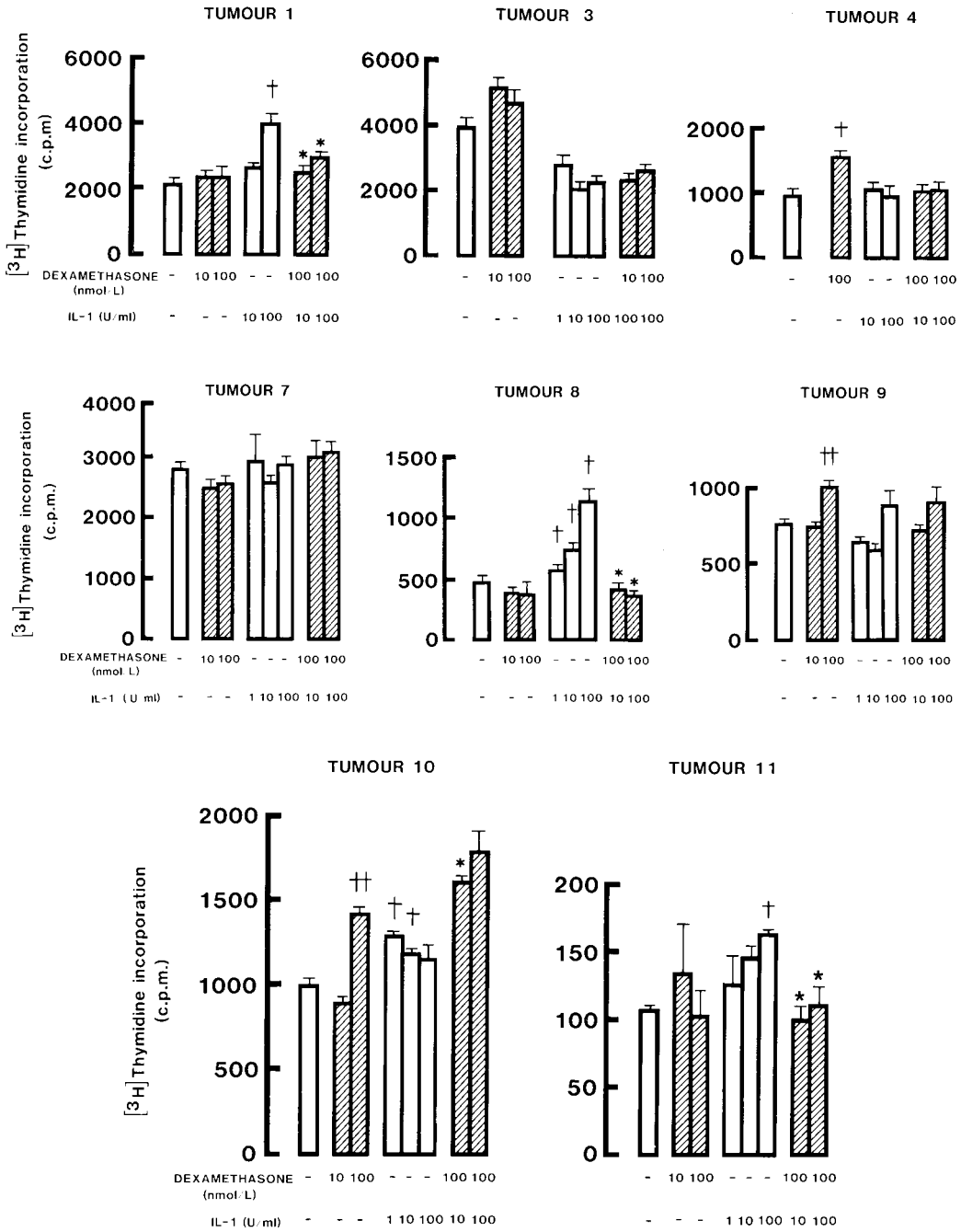

Fig. 3—Effect of IL-1 and dexamethasone on [^{3}H]thymidine incorporation into cultured human meningioma cells. The results are the mean ± SEM for triplicate wells. †$P<0{\cdot}05$, ††$P<0{\cdot}005$ for IL-1 and dexamethasone effects; *$P<0{\cdot}05$, **$P<0{\cdot}005$ for dexamethasone inhibitory or stimulatory effect on IL-1 stimulated [^{3}H]thymidine incorporation

specific enzyme reaction. The detection limit for the assay is 4 U/ml and the coefficient of variation is 8·3 per cent.

Reagents

Recombinant human IL-1α was a gift from Hoffmann-La Roche Inc., Nutley, New Jersey, U.S.A. Human IL-6 was from Boehringer Mannheim; dexamethasone, cholera toxin, forskolin, and dibutyryl cyclic AMP were all from Sigma.

Statistical analysis

Significances were calculated using paired Student's *t*-tests.

RESULTS

Expression of IL-6 mRNA in human meningiomas varied from negative to extensive strong positivity in virtually all cells (Fig. 1 and Table I). All probe omission

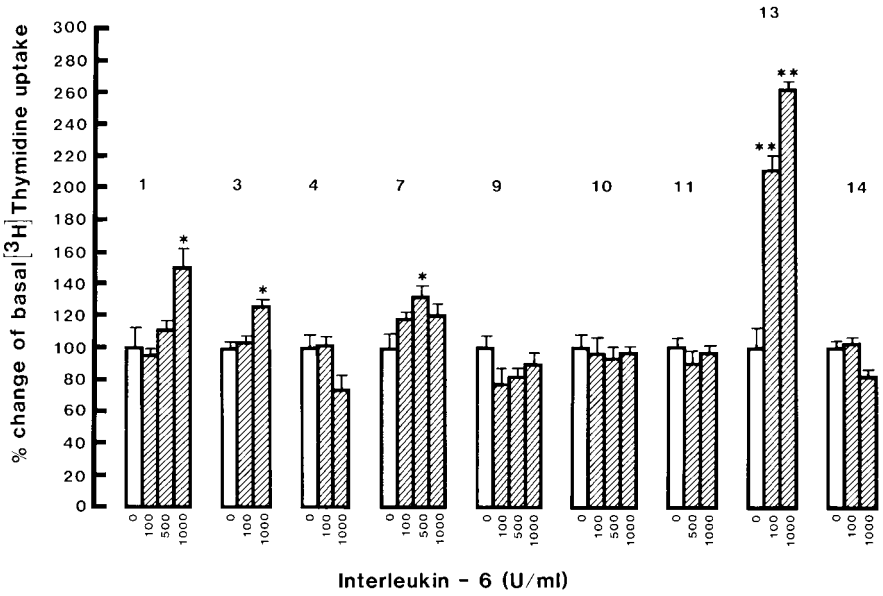

Fig. 4—Effect of IL-6 on [^{3}H]thymidine incorporation into cultured human meningioma cells. The results are the mean ± SEM for triplicate wells. *$P<0{\cdot}05$; **$P<0{\cdot}005$

controls were negative. Normal brain sections were negative for IL-6 transcripts. Sections of all of the meningiomas were examined for inflammatory cells, with very few being identified (<1 per cent). IL-6 protein expression correlated with mRNA expression in most but not all cases (Table I). The discordance between the level of message and protein expression in some tumours could be due to a post-transcriptional down-regulation in protein synthesis for IL-6 or to an increase in IL-6 breakdown. There was also discordance in some cases between basal levels of IL-6 secreted as determined by ELISA and mRNA expression. The secretion of IL-6 may in some cases originate from non-tumour cells which are co-cultured from the original tissue sample. Alternatively, growth factors in the culture medium may affect IL-6 secretion by some tumours. Final IL-6 levels in culture medium also depend on the growth rate of tumour cells and therefore the number of cells per well at the end of the experiment.

Meningiomas with high levels of IL-6 mRNA (Men 3, 7, 8, 11, and 14) produced increased levels of secreted IL-6 in response to IL-1 stimulation (Table I). This is suggestive of a post-translational regulatory mechanism whereby IL-1 signalling in the tumour cells increases translation of the IL-6 message. Dexamethasone efficiently suppressed the IL-6 response to IL-1 in all the cases studied (Fig. 2).

Meningiomas expressing the IL-6 transcript showed a general correlation between IL-1-stimulated tumour growth and the extent of IL-1-induced IL-6 secretion (Table II). Thus, with the exception of Men 1, meningiomas which gave only modest increases in IL-6 secretion in response to IL-1 did not show any measurable increase in growth with exogenous IL-1 at the levels tested, but did respond to exogenous IL-6 with an increase in growth (Figs 3 and 4). In contrast, meningiomas with high levels of IL-6 mRNA that gave the greatest induction of IL-6 secretion in response to IL-1 (Men 8 and 11, Men 14) also had a positive growth response to IL-1 stimulation (Table II and Figs 2 and 3) (Men 14 not tested). Meningiomas with the highest levels of endogenous mRNA for IL-6 (Men 11 and 14) did not, however, give a growth response to exogenous IL-6 (Fig. 4). Dexamethasone, which has been shown to inhibit the IL-6 response to IL-1, also inhibited this IL-1 growth stimulation (Fig. 3). Interestingly, the three meningiomas tested which showed growth stimulation in dexamethasone (Men 4, 9, and 10) were the same three tumours that were not growth-stimulated by either exogenous IL-6 or IL-1; in fact, Men 10 was actually growth-inhibited by IL-1. Meningiotheliomatous and transitional meningiomas seem to be high IL-6 expressers. The effect of IL-1 and dexamethasone in the tumours tested was not affected by the gender of the patient.

In seven of 11 IL-6-secreting meningiomas studied, neither cholera toxin, forskolin, nor dibutyryl cyclic AMP had any effect on IL-6 secretion (Table III). In Men 3, forskolin and dibutyryl cyclic AMP produced a small but significant increase in IL-6 release (Table III). This tumour was one of the more responsive meningiomas to IL-1 stimulation of IL-6 release. Men 13 responded to both cholera toxin and forskolin with a rise in IL-6 release; this tumour was not sensitive to IL-1. However, in Men 2, cholera toxin and forskolin inhibited basal IL-6 secretion, but dibutyryl cyclic AMP had no effect (Table III). Fetal calf serum stimulated IL-6 release in three tumours studied but the response varied between cell cultures (Table IV).

DISCUSSION

IL-6 is synthesized and released by the majority of human meningiomas; in some cultures it stimulated cell proliferation, but in others it had no effect. Such

Table III—Effect of cholera toxin, forskolin, and dibutyryl cyclic AMP on IL-6 secretion from cultured meningioma cells. Data expressed as the mean ± SEM for triplicate samples

	Tumour No.										
	1	2	3	7	8	9	10	11	12	13	14
Basal	340	362 ± 16	140 ± 5	9·9 ± 1·2	10·8 ± 1·1	91 ± 11·5	33 ± 1·5	13·8 ± 1·6	53·5 ± 2·8	76·7 ± 3·3	59 ± 5·2
Cholera toxin, 100 pg/ml	319	213 ± 7**	150 ± 10	8·1 ± 0·3	10·1 ± 4·2	99·4 ± 3	35·3 ± 1·2	8·4 ± 0·3*	52·7 ± 3·5	113·3 ± 10·9*	68 ± 9·2
Forskolin, 10 μmol/l	—	215 ± 5**	198 ± 3*	8·4 ± 0·3	13·5 ± 4·5	92·0 ± 3·5	32 ± 1·1	9·3 ± 1·4*	41·7 ± 0·9*	103 ± 2·9*	62 ± 3·1
Dibutyryl cAMP, 100 nmol/l	345 ± 1	380 ± 21	183 ± 2*	11·4 ± 1·3	13 ± 2·6	116·8 ± 11·5	35·7 ± 1·2	8·5 ± 0·7*	58·3 ± 1·8	—	36·7 ± 2·9**

*P < 0·05.
**P < 0·005.

Table IV—Effect of fetal calf serum (FCS) on IL-6 production*

Meningioma	− FCS	+10% FCS
1	67·8 ± 7·5 (n=3)	443·9 ± 52·9 (n=5)
2	153 ± 36·6 (n=3)	322·7 ± 27·4 (n=2)
3	10 ± 0·9 (n=3)	109 ± 16·5 (n=3)

*The results are cumulated from different experiments each performed in triplicate on three of the meningiomas. Data are expressed as the mean ± SEM. n=number of experiments. The duration of each incubation was 72 h.

variations in response to IL-6 have been reported in previous studies,[17,18] and we confirm this, not only with respect to the IL-6 growth response but also with other parameters. There were significant differences in the secretory and growth responses to IL-1 and dexamethasone between these tumours. Although the *in vivo* effects of IL-1 and IL-6 on growth and of IL-1 on IL-6 secretion are unknown, extrapolation of these *in vitro* results suggests that the loss of IL-6 inhibitory action and of IL-1 control of tumour IL-6 production could occur and could have biological consequences for the tumour.

Some meningiomas expressed high levels of IL-6 transcript which correlated with their ability to increase output of secreted IL-6 in response to IL-1 stimulation. This IL-6 response to IL-1 could be due to an increase in IL-6 transcription via the action of IL-1 on the multiresponse element of the IL-6 promoter. It is interesting to note that only tumours which were already transcribing the IL-6 gene responded to IL-1 in this way. A further possibility is that IL-1 may induce a post-transcriptional activation of IL-6 production. It is unlikely that all of the meningiomas in which IL-1 failed to elicit an increase in IL-6 production are totally refractory to IL-1, since some of these tumours gave either a positive or a negative growth response to IL-1.

These *in vitro* studies show that meningiomas have a varied response to exogenous cytokines IL-1, IL-6 and to dexamethasone. However, the majority of meningiomas that did respond to these cytokines gave a positive growth response either directly to exogenous IL-6 or indirectly from an IL-1 stimulation producing the IL-6. The stimulatory effect of IL-1α observed in this study contradicts the inhibitory effect of IL-1β reported by Boyle-Walsh *et al.*[18] Some of the meningiomas that did not have a positive growth response to either IL-1 or IL-6 were stimulated to proliferate *in vitro* by dexamethasone. This finding has important implications for the treatment of meningiomas. IL-1 synthesized in the astrocytes under the modulation of glucocorticoids controls the production of IL-6, which in turn regulates tumour–host interactions. The results of this study indicate that dexamethasone may no longer play an inhibitory role for meningiomas in which this control by IL-6 is lost. The prognostic and therapeutic significance of these findings needs to be determined.

As in many other cell types, fetal calf serum stimulated IL-6 release in those meningiomas studied. This stimulation is likely to be due to a number of factors in the serum including lipopolysaccharide, which is known to be present. For this reason, we used a low concentration of fetal calf serum (2 per cent) in these experiments. Raising intracellular cyclic AMP levels with forskolin or cholera toxin had no effect on IL-6 release in the majority of tumours. In all but one tumour, dexamethasone inhibited IL-1-stimulated IL-6 release when present and inhibited basal IL-6 secretion in half of the tumours studied. These findings suggest that the control of IL-6 production differs between individual meningiomas.

In conclusion, in some meningiomas, IL-1, IL-6, and glucocorticoids can affect cell growth in culture as assessed by the measurement of thymidine uptake, but whether or not they have a role or roles in meningioma pathogenesis is not at present clear. Several peptide growth factors have also been shown to influence meningioma cell growth, but the importance of each individual factor *in vivo* is not evident. Complex interactions exist between growth factor, cytokines, and steroids, which may act to produce the final growth response of the tumour. Further knowledge of these mechanisms may potentially lead to the development of drugs which can be used to treat those patients with inoperable or recurrent tumours for which, at present, there is no available therapy. The results presented here suggest that the effect of dexamethasone may differ between meningiomas and may be dependent on the responsiveness of the tumours to other cytokines. As this drug is used commonly in the treatment of meningiomas and other brain tumours to reduce oedema in surrounding brain tissue, it is important to clarify whether or not it has a stimulatory or inhibitory effect on growth.

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