Macrophage migration inhibitory factor expression is increased in pituitary adenoma cell nuclei

M E Pyle, M Korbonits, M Gueorguiev, S Jordan[1], B Kola, D G Morris, A Meinhardt[2], M P Powell[3], F X Claret[4], Q Zhang[4], C Metz[5], R Bucala[6] and A B Grossman

Endocrine Oncology, Department of Endocrinology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK

[1]Department of Histopathology, St Bartholomew's Hospital, London EC1A 7BE, UK

[2]The Justus-Liebig-University of Giessen, Germany

[3]National Institute for Neurology and Neurosurgery, London, UK

[4]MD Anderson Cancer Center, Department of Molecular Therapeutics, Houston, Texas, USA

[5]Picower Institute for Medical Research, New York, USA

[6]Yale University School of Medicine, New Haven, Connecticut, USA

(Requests for offprints should be addressed to A B Grossman; Email: A.B.Grossman@qmul.ac.uk)

Abstract

Macrophage migration inhibitory factor (MIF) is an essential regulator of the macrophage responses to endotoxin. MIF also has the ability to override the anti-inflammatory actions of glucocorticoids during an immune response, and is thus an important pro-inflammatory factor. The presence of MIF in cells of the anterior pituitary has been described, and high levels of MIF in other rapidly proliferating tissues have also been demonstrated. It has been hypothesised that MIF release from these cells is influenced by the hypothalamo–pituitary–adrenal axis, and that ACTH and MIF are released simultaneously to exert counter-regulatory effects on cortisol. However, another intracellular role for MIF has also been suggested as it has been shown that MIF exerts an effect on the inhibitory cell cycle control protein p27 through an interaction with Jab1, a protein implicated in p27 degradation.

We studied MIF expression in different normal and adenomatous human pituitary samples using immunohistochemistry and RT-PCR. There was evidence of co-immunoprecipitation of MIF with Jab1, suggesting an interaction of the two proteins. Our results showed that there is increased expression of MIF protein in the nuclei of all pituitary adenomas compared with normal tissue ($P=0{\cdot}0067$), but there was no statistically significant difference in nuclear MIF expression between the different adenoma types. Nuclear MIF expression correlated positively with p27 and its phosphorylated form in normal tissue ($P=0{\cdot}0028$ and $P<0{\cdot}0001$); however, this relationship was not seen in the adenoma samples. Cytoplasmic expression of MIF was found to be variable both in normal and adenomatous samples, with no consistent pattern. MIF mRNA was demonstrated to be present in all tumour and normal samples studied. Somatotroph tumours showed higher MIF mRNA expression compared with normal pituitary or other types of adenomas.

In conclusion, MIF is expressed in cell nuclei in pituitary adenomas to a greater extent than in normal pituitary tissue. We speculate that it may play a role in the control of the cell cycle, but whether its higher level in adenomas is a cause or a consequence of the tumorigenic process remains to be clarified.

Journal of Endocrinology (2003) **176,** 103–110

Introduction

Macrophage migration inhibitory factor (MIF) was first identified in 1966 as a lymphokine, released specifically from activated T lymphocytes in response to antigenic or mitogenic stimulation (David 1966, Weiser *et al.* 1989), where it was thought to provide a mechanism whereby macrophages could be localised to sites of delayed hypersensitivity reactions (Bloom & Bennett 1966). Further research then demonstrated that monocytes and macrophages themselves are also important sources of MIF production (Calandra *et al.* 1994, 1998). More recently, MIF has been shown to be an essential regulator of the macrophage response to endotoxin, in particular sustaining macrophage survival and up-regulating the Toll-like receptor 4 (TLR4), the signal-transducing molecule of the lipopolysaccharide receptor complex (Roger *et al.* 2001).

0022–0795/03/0176–103
Online version via http://www.endocrinology.org

However, MIF has also been implicated in neuroendocrine regulation: it is present in and released from anterior pituitary cells in response to endotoxin stimulation (Bernhagen *et al.* 1993). MIF localises to granules present exclusively in adrenocorticotrophin (ACTH)- and thyrotrophin-secreting cells in the normal anterior pituitary gland in mice (Nishino *et al.* 1995) and is present in human corticotroph adenomas (Tampanaru-Sarmesiu *et al.* 1997). Furthermore, it was reported that this MIF could be released from the anterior pituitary *in vitro* in response to corticotrophin-releasing hormone as well as by endotoxin *in vivo* (Nishino *et al.* 1995). In addition, leukocyte MIF production is *increased* by corticosteroids, while recombinant MIF over-rides glucocorticoid-induced inhibition of a number of cytokines, including tumour necrosis factor α and interleukin (IL)-1β, IL-6 and IL-8. The concept has therefore arisen that pituitary-derived MIF counter-regulates the hypothalamo–pituitary–adrenal axis, and thus maintains homeostasis in an organism during severe inflammatory reactions associated with trauma or infection. In experiments performed on rats, endotoxin, a known stimulator of the hypothalamo–pituitary–adrenal axis, was shown also to cause a rise in circulating MIF which appeared to be pituitary-dependent (Bernhagen *et al.* 1993, 1998). It was therefore postulated that MIF may be released from the human pituitary alongside ACTH, but while ACTH stimulates cortisol secretion, MIF has opposite effects on inflammatory reactions at a cellular level (Nishino *et al.* 1995). MIF present in the corticotroph cells has also been postulated to modulate the release of ACTH (Tampanaru-Sarmesiu *et al.* 1997).

However, we have previously studied variations in MIF levels in humans under conditions of ACTH stimulation or suppression (Isidori *et al.* 2002). This study suggested that circulating MIF in the human was not, to any major extent, derived from the pituitary, suggesting that pituitary-derived MIF may have a function predominantly *within* the pituitary. Recently, data have appeared compatible with this idea (Kleemann *et al.* 2000). Kleemann *et al.* suggested that MIF might play a crucial intracellular role in the control of the cell cycle by co-localising with Jun activation domain binding protein 1 (Jab1) (see Claret *et al.* 1996) in the cell cytosol to form a complex. MIF then antagonises Jab1-dependent cell-cycle regulation by increasing $p27^{Kip1}$ (p27) expression through stabilisation of the p27 protein. Consequently, the Jab1-mediated rescue of fibroblasts from growth arrest is blocked by MIF. The authors postulated that MIF may act broadly to negatively regulate Jab1-controlled pathways, and that this MIF–Jab1 interaction may provide a molecular basis for key activities of MIF. Thus, at least part of the function of MIF would be independent of any circulating anti-glucocorticoid role. The purpose of this study was, therefore, to investigate the localisation of MIF within the human pituitary, in both normal pituitary as well as in a number of different functioning and non-functioning pituitary adenomas. Furthermore, MIF was correlated with the pituitary content of p27, phosphorylated p27 (P-p27) and Jab1, and the proliferation marker Ki-67.

Materials and Methods

For immunohistochemical studies, pituitary samples were collected at transsphenoidal surgery ($n=50$) and consisted of non-functioning pituitary adenomas (NFPA, $n=18$), ACTH-secreting tumours ($n=12$), growth hormone (GH)-secreting tumours ($n=13$) and prolactin-secreting tumours ($n=7$) (Table 1). Normal pituitaries were used as controls. To avoid inconsistency in the handling of tissue samples which is inevitable with autopsy controls, the control normal pituitaries ($n=10$) were part of resection specimens removed at transsphenoidal surgery for presumptive tumours that proved on staining to consist of normal pituitary tissue and architecture; these generally provide better control tissues compared with autopsy material, as the material is prepared in an identical manner to the tumour tissue. Our previous studies utilising these controls have provided data compatible with studies using autopsy controls (Kovacs *et al.* 2001). All samples had been classified histologically at the time of surgery by a consultant histopathologist using haematoxylin and eosin, immunohistochemical and reticulin staining. For RT-PCR studies, 30 pituitary adenoma samples were studied for MIF mRNA expression: 6 ACTH-secreting, 7 GH-secreting, 6 prolactin-secreting and 11 non-functioning adenomas. In this case, pituitaries removed at autopsy were of necessity used as controls ($n=13$). All studies were approved by our institutional review board ethical committee.

Immunohistochemistry

Immunohistochemical analysis of the samples was carried out using the Avidin Biotin Complex (ABC) immunoperoxidase system. Both monoclonal and polyclonal rabbit anti-mouse MIF antibodies raised against the full-length human MIF molecule were used in 1:100 (polyclonal) and 1:1000 (monoclonal) dilution. The specificity of the primary antibody staining was investigated by pre-incubation with human MIF as a blocking peptide. Western blotting showed a band of the expected size (data not shown). All pituitary sample sections were analysed for MIF monoclonal and MIF polyclonal staining at × 630 magnification using a bright field Leica microscope (Leica Microsystems Ltd). In each section, approximately 500 cells were analysed in areas throughout the tumour field picked at random. In any one field, an 'S' shape was traced across the field and all cells along this shape were analysed and the results recorded without any prior knowledge of the tumour type. Staining intensity in individual cells was

Table 1 Clinical details of patients studied with immunohistochemistry

	Age (years)	**Sex**	**Immunostaining**
Clinical diagnosis			
Acromegaly	33	M	GH
Acromegaly	49	F	GH, PRL
Acromegaly	45	M	gh
Acromegaly	54	M	GH, prl, acth
Acromegaly	33	M	GH, acth
Acromegaly	39	M	GH, prl, alpha-hcg
Acromegaly	29	F	GH
Acromegaly	43	M	GH, PRL
Acromegaly	32	F	GH
Acromegaly	49	F	GH, PRL
Acromegaly	50	F	GH, prl
Acromegaly	53	M	GH
Acromegaly	70	M	GH
Prolactinoma	25	F	PRL
Prolactinoma	21	M	PRL
Prolactinoma	37	F	PRL, alpha-hcg
Prolactinoma	67	F	PRL
Prolactinoma	29	F	PRL
Prolactinoma	61	M	PRL
Prolactinoma	28	F	PRL
Cushing's disease	17	F	ACTH
Cushing's disease	52	F	ACTH
Cushing's disease	51	F	ACTH
Cushing's disease	64	M	ACTH
Cushing's disease	51	F	ACTH
Cushing's disease	26	F	ACTH
Cushing's disease	34	F	ACTH
Cushing's disease	41	F	ACTH
Cushing's disease	31	F	ACTH
Cushing's disease	57	F	ACTH
Cushing's disease	64	M	ACTH
Cushing's disease	19	M	ACTH
NFPA	64	M	alpha-hcg
NFPA	36	F	All negative
NFPA	65	M	prl, alpha-hcg
NFPA	65	F	prl, alpha sub-unit
NFPA	53	F	All negative
NFPA	40	F	All negative
NFPA	78	M	alpha sub-unit
NFPA	70	F	alpha sub-unit
NFPA	36	M	gh, prl
NFPA	67	M	All negative
NFPA	74	M	fsh, lh
NFPA	65	F	All negative
NFPA	50	M	All negative
NFPA	70	M	alpha-hcg
NFPA	58	F	All negative
NFPA	72	M	fsh, lh, alpha-hcg
NFPA	69	F	All negative
NFPA	50	M	All negative

Hormone names in small letters suggest low level of hormone staining.
PRL, prolactin; hCG human chorionic gonadotrophin;
FSH, follicle-stimulating hormone; LH, luteinizing hormone.

recorded as either positive or negative staining. The numbers of cells falling into each category were then expressed as a percentage of the total 500 cells counted. The staining intensity in both the cell nuclei and the cytoplasm was analysed.

Correlations were performed using previous data obtained on p27, P-p27, Jab1 and Ki-67 staining (Korbonits *et al.* 2002).

Co-immunoprecipitation

Tissue from a human pituitary adenoma was ground up and lysed in MCL buffer (20 mM Tris–HCl, pH 7·5, 150 mM NaCl, 0·5% NP-40, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulphonylfluoride), supplemented with protease inhibitors (leupeptin 10 μg/ml; pepstatin 2 μg/ml; antipeptin 50 μg/ml; aprotinin 2 mg/ml; chymostatin 20 μg/ml; benzamidine 2 μg/ml) and phosphatase inhibitors (2 mM Na-fluoride, 1 mM Na-orthovanadate, 10 mM β-glycerophosphate) for 30 min at 4 °C. After centrifugation at 4 °C, 300 μg total lysates were subjected to immunoprecipitation with either a rabbit polyclonal anti-human MIF or a non-immune serum (NIS) as a control, for 2 h at 4 °C. Protein A-sepharose CL4B beads (Amersham-Pharmacia-Biotech, Piscataway, NJ, USA) were then added and rotated for an additional 2 h at 4 °C. Immune complexes were collected by centrifugation, and washed three times in MCL buffer. Proteins were resolved on 12% SDS-PAGE and detected by immunoblotting with an anti-Jab1 antibody (Zymed Laboratories, San Francisco, CA, USA). The reaction was detected with enhanced chemiluminescence (ECL PLUS) reagents (Amersham-Pharmacia-Biotech).

RT-PCR

Tissue extraction and cDNA synthesis were performed as previously described (Korbonits *et al.* 2001*b*). PCR reactions containing the intron-spanning MIF primers were performed followed by duplex PCR reactions to determine the relative expression of MIF using the MIF primers as well as the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), according to a previously validated semi-quantitative technique (Edwards & Gibbs 1994, Jacobs *et al.* 1997, Korbonits *et al.* 2001*a*). cDNA (2·5 μl; 250 ng RNA equivalent) was used as template, together with 200 μmol/l deoxynucleotides (Promega), 0·5 μmol final concentration of MIF primers (sense 5′TTCCTCTCCGAGCTCACC3′ and antisense 5′CGTTTATTTCTCCCCACCAG3′, giving rise to a 399 basepair PCR product) and 0·1 μmol final concentration of GAPDH primers (in the duplex reactions (Korbonits *et al.* 1998)), 1·5 mmol/l $MgCl_2$, 0·125 U Taq (Promega), and TaqStart antibody (Clontech, Heidelberg, Germany) in a 25 μl PCR reaction. PCR cycles were performed 35 times for the MIF only and 25 times for the duplex PCR at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min after a denaturing cycle of 95 °C for 5 min. For every batch of PCR reaction, a negative control tube was run with water instead of cDNA. Each pituitary RNA sample had a control RT reaction where the RT enzyme

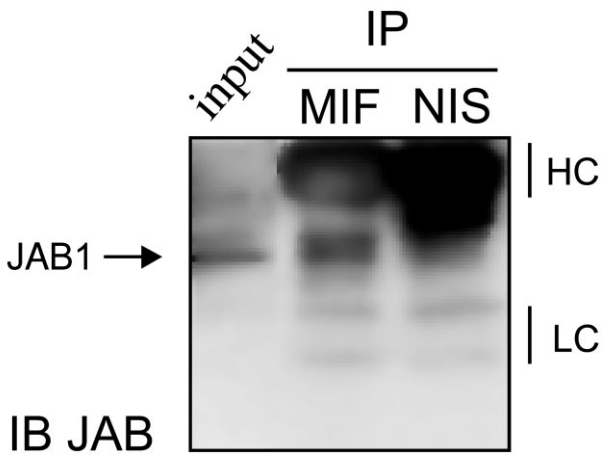

Figure 1 Co-immunoprecipitation of MIF with Jab1. A human pituitary adenoma lysate was prepared in non-denaturating conditions and the proteins were subjected to co-immunoprecipitation (IP) with either specific MIF antibodies or a non-immune serum (NIS) as a control. MIF-associated proteins were 'pulled down' with protein A-Sepharose beads, separated on SDS-PAGE and analysed by immunoblot (IB) using an anti-Jab1 antibody. An input representing 10% of the total lysate is shown. HC, heavy chain immunoglobulin; LC, light chain immunoglobulin.

was not added to the mixture. These 'control' samples did not show amplification on subsequent PCRs. The duplex PCRs were performed prior to the plateau phase of the synthesis curve for both genes, and the cycling curve with the MIF and GAPDH primers showed parallel amplification. The PCR products were run on 2% ethidium bromide-stained agarose gels, which were photographed and analysed by a Kodak DC120 camera and the Kodak Image Analysis software version 3.5.0, and expressed as optical density units. A ratio between MIF and GAPDH was obtained for each individual sample.

Statistical analysis

The Shapiro–Wilk test of non-normality was performed on all data sets and all were found to be non-normally distributed. Therefore, non-parametric statistical tests were applied to all the data sets, as appropriate (Spearman rank correlation, Mann Whitney U test and the Kruskal–Wallis test). All data are given as mean values ± S.E.M. The level of statistical significance was taken as $P<0{\cdot}05$.

Results

The co-immunoprecipitation study revealed that the immunoprecipitate for MIF was co-expressed in the same position as the Jab1 immunoblot (Fig. 1), suggesting a probable interaction of these two peptides. MIF mRNA and protein were found to be expressed in both normal and adenomatous pituitaries. With regard to protein expression, this was localised to both nuclei and cytoplasm, but was particularly strongly expressed in the cell nucleus (Fig. 2A). The staining was specific as it was blocked by preincubation with MIF antiserum (Fig. 2B). Analysing the nuclear staining quantitatively, there was noted to be more MIF protein present in the adenomatous samples compared with the normal pituitary. With the monoclonal antibody, 45 ± 3% of the cells showed positive nuclear staining for MIF while only 26 ± 5% was seen in the normal pituitary sample ($P=0{\cdot}0017$); with the polyclonal antibody, 59 ± 7% of the cells in adenomas showed nuclear staining compared with 35 ± 6% of the cells in the normal pituitary sample ($P=0{\cdot}0067$), therefore showing a similar ratio ($\sim 1{\cdot}7$) between the adenoma and the normal samples using either antibody (Fig. 3). Examining the individual tumour types, there was no significant difference between the tumours (Kruskal-Wallis test: 0·22 and 0·06 for the monoclonal and polyclonal antibodies respectively) (Fig. 4). MIF nuclear protein expression correlated positively with both nuclear p27 and P-p27 in the normal pituitary samples ($P=0{\cdot}0028$ and $P<0{\cdot}0001$ respectively); however, there was no statistically significant correlation between MIF and either p27 or P-p27 expression in the pituitary adenomas. Correlations between MIF and Jab1 or Ki-67 staining were not significant for either the normal or the adenomatous samples.

In terms of cytoplasmic staining, variable amounts of cytoplasmic positivity were observed within a sample and between different tissue types. In general, the cytoplasmic staining was proportional to the nuclear staining, but there were no statistically significant differences between normal and adenomatous pituitary, or between different tumour types.

Study of MIF mRNA expression by semi-quantitative RT-PCR confirmed the presence of MIF in all samples studied. There was a significantly higher expression of MIF mRNA in samples from GH-secreting tumours compared with either normal pituitary or other types of pituitary tumour, but there was no difference between normal tissue and the other tumour types (Fig. 5).

Discussion

MIF was expressed both in normal pituitary and in all pituitary adenomas, as demonstrated by both immunohistochemistry and RT-PCR, while co-precipitation studies suggested an interaction with Jab1 protein. There was a statistically significant difference in the amount of MIF protein expressed in cell nuclei comparing normal pituitaries and adenomas, with adenomas expressing more nuclear MIF – this was shown by both the monoclonal antibody and the polyclonal antibody. MIF expression appears to be increased in the adenoma group as a whole; however, there was no difference in MIF immunostaining between the individual pituitary adenoma types. The

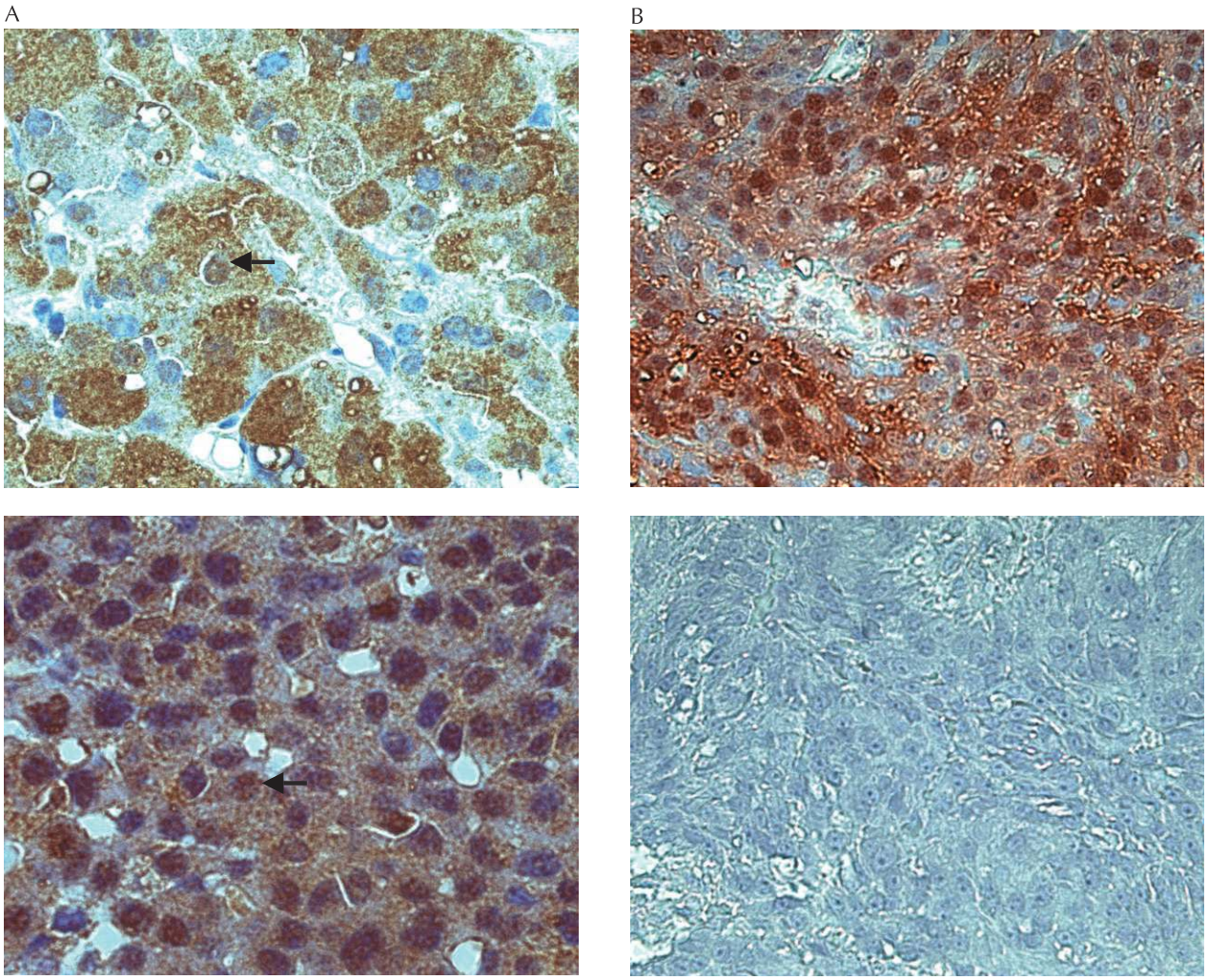

Figure 2 (A) Normal pituitary (top) and corticotrophinoma (below) immunostained with polyclonal rabbit MIF antibody. Note that there is significant nuclear immunostaining both in the normal pituitary and, particularly, in the corticotrophinoma; some positive-staining nuclei are shown by black arrows. There is also patchy cytoplasmic immunostaining in the normal pituitary and, more uniformly, in the tumour. (B) MIF staining in normal human pituitary. In the top panel, positive MIF staining in normal pituitary is evident, while in the bottom panel this is absent in a slide pre-treated with human recombinant MIF protein. Magnification × 1000.

results of the semi-quantitative RT-PCR did not show increased MIF mRNA expression in adenomas as a group, although there was a specific increase in expression in GH-secreting tumours alone.

Previous studies have demonstrated the presence of MIF protein in normal pituitary tissue, ACTH-secreting and non-functioning adenomas (Tampanaru-Sarmesiu *et al.* 1997), and we now confirm its presence in prolactin-secreting and GH-secreting adenomas as well. However, the novel and unexpected finding in the present study is the clear and marked demonstration of MIF nuclear staining, and its selective increase in pituitary tumours. This may suggest that it is playing some role in either the process of tumorigenesis, or as a response to this process. In other tissues, high levels of MIF protein have previously been reported in rapidly proliferating tissue, such as the lens of the eye during experimental cataract, and of MIF mRNA in carcinoma of the prostate when compared with normal prostate tissue (Meyer-Siegler & Hudson 1996). It has been suggested that MIF exerts a pro-proliferative effect by stimulation of prostaglandin and leukotriene synthesis (Mitchell *et al.* 1999). Glucocorticoids are known to inhibit cell proliferation and to induce differentiation (Rogatsky *et al.* 1997), and therefore inhibition of the effect of cortisol induced by MIF could induce cell proliferation. Alternatively, Kleemann *et al.* (2000) have

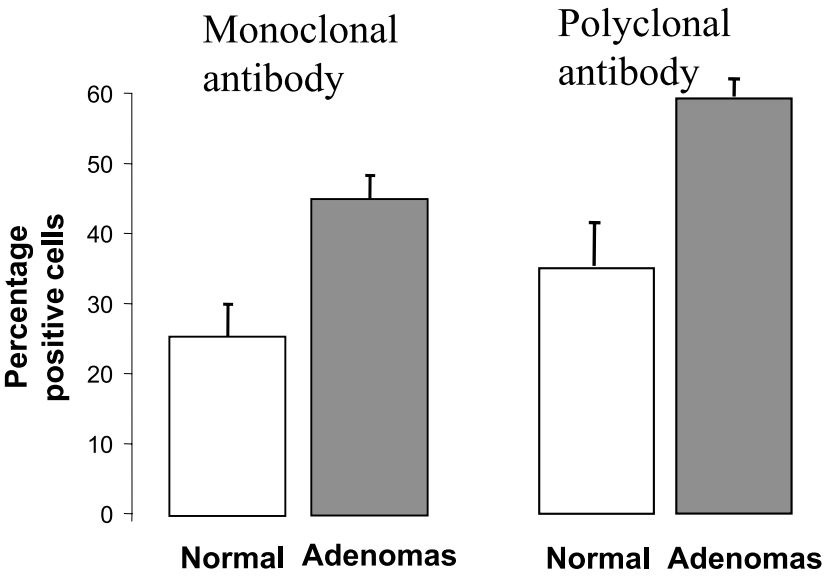

Figure 3 A comparison of MIF staining in normal ($n=10$) and adenomatous ($n=50$) samples with monoclonal and polyclonal MIF antibodies (means ± S.E.M.). With both antibodies nuclear MIF is seen to be higher in the adenomatous samples.

suggested that cytoplasmic MIF inhibits Jab1 (a stimulator of cell proliferation, possibly via stimulating the degradation of the cell cycle inhibitor p27), thus preventing p27 degradation. This promotes the effect of p27, which is to prevent cell proliferation or tumour growth (Kleemann *et al.* 2000). However, we were unable to demonstrate changes in cytoplasmic MIF. We did, however, show that MIF immunoprecipitates with Jab1, suggesting that some form of interaction does take place. It is unclear as to whether increased nuclear MIF *blocks* Jab1 (thus suppressing growth) or, by sequestering it within the nucleus, *enhances* Jab1 activity, hence promoting tumour growth.

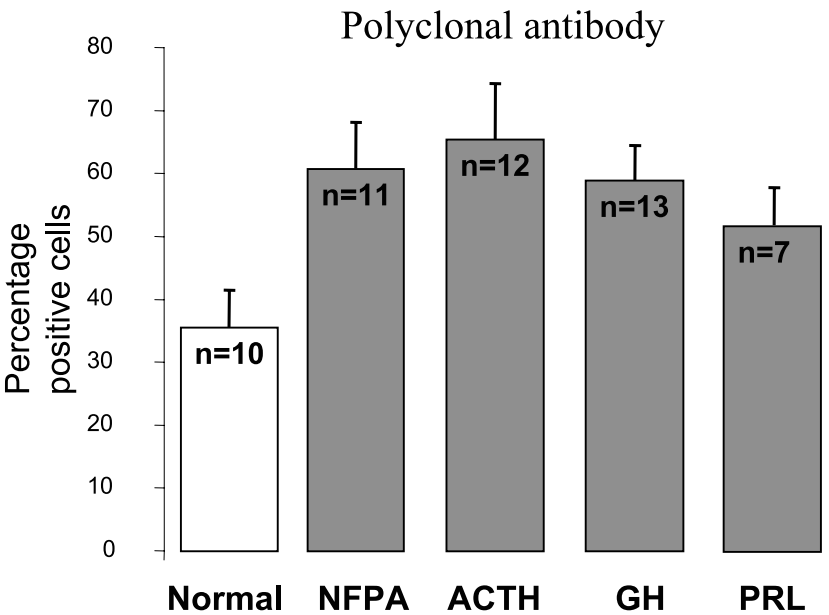

Figure 4 A comparison of MIF staining between the various pituitary adenoma types obtained with the polyclonal antibody (means ± S.E.M.). There was no statistical difference between the different tumour types.

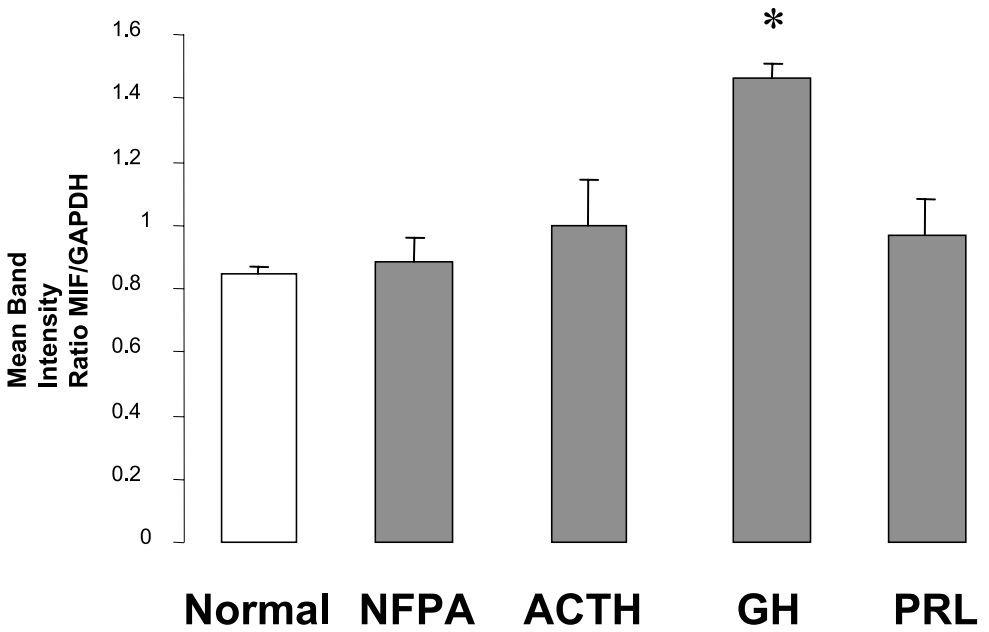

Figure 5 Relative expression of MIF mRNA compared with the house-keeping gene, GAPDH, in normal and abnormal pituitary tissue (means ± S.E.M.). *$P<0{\cdot}05$ compared with the other pituitary samples.

Cytoplasmic MIF did not show statistically significant changes between normal and abnormal tissue, but in general it appeared to correlate directly with nuclear MIF. This suggests that changes in nuclear MIF are unlikely to represent a change in compartmentation. In either case, the dominant control of MIF appears to be post-transcriptional, except possibly in GH-secreting tumours.

In normal pituitary tissue there was a significant positive correlation between MIF and both p27 and P-p27. p27 is phosphorylated to P-p27 on threonine 187 which is then subject to ubiquitination in the SCF complex and proteasomal degradation. Previous studies have shown that p27 is generally decreased in pituitary tumours, as is P-p27. However, the correlation between MIF and both p27 and P-p27 is lost in the adenomas, where nuclear MIF specifically increases. We hypothesised that the lack of correlation of p27 and MIF could be related to the presence of the data from the ACTH-secreting tumours, known to have very low p27 levels (Lidhar *et al.* 1999). However, even after removing the ACTH-secreting tumour data and re-calculation, there was still no significant correlation seen. In this study, there was no significant correlation between MIF and Jab1 protein levels in either normal or abnormal tissue. Similarly, while the protein expression of p27 is clearly differentially expressed in normal versus adenomatous pituitary tissue, this was *not* found to be the case for Jab1 (Korbonits *et al.* 2002). It is therefore possible that intra-nuclear MIF has some other role in the control of the cell cycle, independent of any association with Jab1, but possibly relating to p27 regulation.

In conclusion, this study has demonstrated that there is increased expression of nuclear MIF protein in pituitary adenomas as compared with normal tissue. This MIF co-immunoprecipitated with the cell cycle regulator, Jab1. It was also found that MIF expression correlated positively with p27 in normal pituitary tissue, but not in adenomatous samples. Our results suggest that MIF may play a role in the control of the cell cycle, but whether its high levels in adenomas is a cause, or a consequence, of the tumoral process, remains to be clarified.

Acknowledgements

M K was supported by the MRC, M G and D G M by the Joint Research Board of St Bartholomew's Hospital, and B K by a grant from the Cancer Research Committee of St Bartholomew's Hospital. F X C was supported by grants from the National Institutes of Health (1RO1CA90853–01).

References

Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, Manogue KR, Cerami A & Bucala R 1993 MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature* **365** 756–759.

Bernhagen J, Calandra T & Bucala R 1998 Regulation of the immune response by macrophage migration inhibitory factor: biological and structural features. *Journal of Molecular Medicine* **76** 151–161.

Bloom BR & Bennett B 1966 Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* **153** 80–82.

Calandra T, Bernhagen J, Mitchell RA & Bucala R 1994 The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *Journal of Experimental Medicine* **179** 1895–1902.

Calandra T, Spiegel LA, Metz CN & Bucala R 1998 Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria. *PNAS* **95** 11383–11388.

Claret FX, Hibi M, Dhut S, Toda T & Karin M 1996 A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. *Nature* **383** 453–457.

David JR 1966 Delayed hypersensitivity *in vitro*: its mediation by cell-free substances formed by lymphoid cell–antigen interaction. *PNAS* **56** 72–77.

Edwards MC & Gibbs RA 1994 Multiplex PCR: advantages, development and applications. *PCR Methods and Applications* **3** S65–S75.

Isidori AM, Kaltsas GA, Korbonits M, Pyle M, Gueorguiev M, Meinhardt A, Metz C, Petrovsky M, Bucala R & Grossman AB 2002 Response of serum macrophage migration inhibitory factor (MIF) levels to stimulation or suppression of the hypothalamo–pituitary–adrenal axis in normal subjects and patients with Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* **87** 1834–1840.

Jacobs RA, Dahia PLM, Satta M, Chew SL & Grossman ABi 1997 Induction of nitric oxide synthase and interleukin-1b, but not heme oxygenase, messenger RNA in rat brain following peripheral administration of endotoxin. *Molecular Brain Research* **49** 238–246.

Kleemann R, Hausser A, Geiger G, Mischke R, Burger Kentischer A, Flieger O, Johannes FJ, Roger T, Calandra T, Kapurniotu A, Grell M, Finkelmeier D, Brunner H & Bernhagen J 2000 Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature* **408** 211–216.

Korbonits M, Jacobs RA, Aylwin SJB, Burrin JM, Dahia PLM, Monson JP, Trainer PJ, Chew SL, Besser GM & Grossman AB 1998 Expression of the growth hormone secretagogue receptor in pituitary adenomas and other neuroendocrine tumors. *Journal of Clinical Endocrinology and Metabolism* **83** 3624–3630.

Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, Kangawa K & Grossman AB 2001*a* The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumours. *Journal of Clinical Endocrinology and Metabolism* **86** 881–887.

Korbonits M, Chitnis MM, Gueorguiev M, Norman D, Rosenfelder N, Suliman M, Jones TH, Noonan K, Fabbri A, Besser GM, Burrin JM & Grossman AB 2001*b* The release of leptin and its effect on hormone release from human pituitary adenomas. *Clinical Endocrinology* **54** 781–789.

Korbonits M, Chahal HS, Kaltsas G, Jordan S, Urmanova Y, Khalimova Z, Harris PE, Claret FX & Grossman AB 2002 Expression of phosphorylated $p27^{Kip1}$ protein and Jab1 (Jun activation domain-binding protein 1) in human pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **87** 2635–2643.

Kovacs K, Rotondo F, Stefaneanu L, Fereidooni F, Horvath E, Lloyd RV & Scheithauer BW 2001 Glucocorticoid receptor expression in nontumorous human pituitaries and pituitary adenomas. *Endocrine Pathology* **11** 267–275.

Lidhar K, Korbonits M, Jordan S, Khalimova Z, Kaltsas G, Lu X, Clayton RN, Jenkins PJ, Monson JP, Besser GM, Lowe DG & Grossman AB 1999 Low expression of the cell cycle inhibitor $p27^{Kip1}$ in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **84** 3823–3830.

Meyer-Siegler K & Hudson PB 1996 Enhanced expression of macrophage migration inhibitory factor in prostatic adenocarcinoma metastases. *Urology* **48** 448–452.

Mitchell RA, Metz CN, Peng T & Bucala R 1999 Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. *Journal of Biological Chemistry* **274** 18100–18106.

Nishino T, Bernhagen J, Shiiki H, Calandra T, Dohi K & Bucala R 1995 Localization of macrophage migration inhibitory factor (MIF) to secretory granules within the corticotrophic and thyrotrophic cells of the pituitary gland. *Molecular Medicine* **1** 781–788.

Rogatsky I, Trowbridge JM & Garabedian MJ 1997 Glucocorticoid receptor-mediated cell cycle arrest is achieved through distinct cell-specific transcriptional regulatory mechanisms. *Molecular and Cellular Biology* **17** 3181–3193.

Roger T, David J, Glauser MP & Calandra T 2001 MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* **414** 920–924.

Tampanaru-Sarmesiu A, Stefaneanu L, Thapar K, Kovacs K, Donnelly T, Metz CN & Bucala R 1997 Immunocytochemical localization of macrophage migration inhibitory factor in human hypophysis and pituitary adenomas. *Archive of Patholology and Laboratory Medicine* **121** 404–410.

Weiser WY, Temple PA, Witek-Giannotti JS, Remold HG, Clark SC & David JR 1989 Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. *PNAS* **86** 7522–7526.

Received 28 July 2002
Accepted 10 September 2002