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David T. Lincoln · Fred Sinowatz
Labiba Temmim-Baker · Hisham I. Baker
Sabine Kölle · Michael J. Waters

Growth hormone receptor expression in the nucleus and cytoplasm of normal and neoplastic cells

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Abstract Growth hormone (GH) exerts its regulatory functions in controlling metabolism, balanced growth and differentiated cell expression by acting on specific receptors which trigger a phosphorylation cascade, resulting in the modulation of numerous signalling pathways dictating gene expression. A panel of five monoclonal antibodies was used in mapping the presence and somatic distribution of the GH receptor by immunohistochemistry in normal and neoplastic tissues and cultured cells of human, rat and rabbit origin. A wide distribution of the receptor was observed in many cell types. Not all cells expressing cytoplasmic GH receptors displayed nuclear immunoreactivity. In general, the relative proportion of positive cells and intensity of staining was higher in neoplastic cells than in normal tissue cells. Immunoreactivity showed subcellular localisation of the GH receptor in cell membranes and was predominantly cytoplasmic, but strong nuclear immunoreaction was also apparent in many instances. Intense immunoreactivity was also observed in the cellular Golgi area of established cell lines and cultured tissue-derived cells in exponential growth phase, indicating cells are capable of GH receptor synthesis. The presence of intracellular GH receptor, previously documented in normal tissues of mostly animal origin, is the result of endoplasmic reticulum and Golgi localisation. Heterogeneity of immunoreactivity was found in normal and neoplastic tissue with a variable range of positive cells. The nuclear localisation of immunoreactivity is the result of nuclear GH receptor/binding protein, identically to the cytosolic and plasma GH-binding protein, using a panel of five monoclonal antibodies against the GH receptor extracellular region. The expression of GH receptors, not only on small proliferating tumour cells such as lymphocytes, but also on well differentiated cells including keratinocytes, suggests that GH is necessary not only for differentiation of progenitor cells, but also for their subsequent clonal expansion, differentiation and maintenance.

D.T. Lincoln (✉)
Department of Medical Science, Faculty of Allied Health, Kuwait University, P.O. Box 31470 Sulaibikhat, 90805 Sulaibikhat, Kuwait
Tel. +9654844187, 48333631; fax +9654844187, 4830937

F. Sinowatz · S. Kölle
Institute of Veterinary Anatomy, University of Munich, Germany

L. Temmim-Baker · H.I. Baker
Kuwait Cancer Control Centre, Kuwait

M.J. Waters
Department of Physiology and Pharmacology, University of Queensland, Australia

Introduction

Growth hormone (GH) has been known for a long time as the major hormone regulating somatic and skeletal growth and bone maturation. It is mitogenic for the cellular stromal elements consisting of fibroblasts, adipocytes, macrophages, endothelial cells and acts directly on erythroblasts and erythroleukaemia cells (Merchav et al. 1988). It modulates the synthesis of multiple mRNA species in mammalian tissues, plays a crucial role in controlling metabolism and acts throughout the body to promote not only balanced growth, but also differentiated cell expression. The processes responsible for this modulation are not fully understood. The original somatomedin hypothesis suggested that the mitogenic actions of GH were mediated by circulating insulin-like growth factors (IGFs), derived from the liver in response to GH, synthesised in the pituitary gland (Salmon and Doughaday 1957). However, recent evidence from animals and developing humans shows that IGF-I is also widely synthesised locally in supporting tissue (Hoyt et al. 1988) and the concept that it may be directly regulated by local action of GH to promote both stem cell differentiation and proliferation in vitro (Stracke et al. 1984) and in vivo (Isgaard et al. 1988) has become firmly established. A local response to cytokines requires the presence and subsequent binding to appropriately located receptors. Cytokines are chemical mediators, which include interleukins, polypeptide hormones and other growth factors,

and they regulate growth, differentiation and specific cellular functions by interacting with their cognate receptors. Thus, the actions of GH on target tissue are mediated through binding to the GH receptors, and involve a phosphorylation cascade that results in the modulation of numerous signalling pathways (Argetsinger et al. 1993). The absence of this cascade leads to dwarfism, as exemplified by Laron-type dwarves expressing defective GH receptor (Godowski et al. 1989; Baumgartner et al. 1994). The GH receptor is a member of the large haematopoietic or cytokine receptor family, which contain no tyrosine kinase domain in their cytoplasmic region. The haematopoietic or cytokine/GH receptor family (Class I) and the interferon receptor family (Class II) share both structural features and newly identified common signal transduction pathways. Furthermore, both classes of receptors are associated with various members of the Janus family of tyrosine kinases and activate a new family of transcription factors, known as signal transducers and activators of transcription, which couple ligand binding to the activation of gene expression (Goffin et al. 1996).

As the instrument of signalling by GH the GH receptor is central to understanding the cellular actions of this pleiotropic hormone. Cloning and sequencing of the GH receptor, to obtain insight into the mechanism of transcriptional regulation by GH, has shown that the receptor is not homologous to other receptors of known function and is homologous only to the prolactin receptor. Little is known about the events leading to biological effects after the binding of GH to its receptor (Leung et al. 1987). Since nuclear association and localisation of receptor molecules and expression of polypeptide-induced transcriptional events have been reported to occur with receptors for steroids (Beato 1989) and prolactin (Buckley et al. 1992), the identification and localisation of specific receptors for GH may point to the likely target tissues for biological action. While mapping the somatic distribution of the GH receptor by immunohistochemistry, a distinct localisation of the receptor in the nucleus of some cells was also observed. In this investigation we have used a panel of monoclonal antibodies (MAbs), directed against the hormone binding side of the receptor, which recognise independent epitopes on the extracellular portion of the receptor and are reactive with the GH-binding protein (GHBP) of human, rabbit and rat species, to immunohistochemically localise GH receptors in the nucleus, cytoplasm and cell membrane of normal and neoplastic cells.

Materials and methods

Normal tissues

Human tissues, collected at autopsy, were obtained from the Royal Brisbane Hospital, the Brisbane City Morgue and the Kuwait Cancer Control Centre. Tissues were routine fixed in 10% buffered neutral formalin and processed. Wistar rats, DA rats and Agouti rabbits were obtained from the Central Animal Breeding House, University of Queensland. Animals, maintained on adlibitum pellet feeding with 12-h light/dark cycles, were anaesthetised by an i.p. injection of pentabarbitone (30 mg/kg body weight) and perfused intracardially with phosphate-buffered saline (PBS, pH 7.4) until blanching, followed by Bouin's solution (0.9% v/v picric acid, 9% v/v formaldehyde, 5% acetic acid). Tissues were dissected and postfixed in Bouin's solution for 4 h at 4 °C. All tissues were embedded in paraffin wax by standard histological procedures. Semi-serial 5-µm sections were collected onto polylysine-coated slides. Normal tissues investigated for GH receptor expression included the olfactory system, the central nervous system, integument, mammary glands (including pregnant and lactating), digestive system, reproductive organs, skeletal and muscular tissue.

Tumour tissues

Tumours were obtained from the Royal Brisbane Hospital, Australia, the Cancer Control Centre, Kuwait and the University of Munich, Germany. The following human tumours were investigated: (1) lymphoma tumours, consisting of B-cell lymphoma, Mycosis fungoides, cutaneous T-cell lymphoma, adult T-cell leukaemia/lymphoma, lymphosarcoma of dog; (2)basalioma; (3) melanoma, consisting of the following histological entities: common melanocytic naevi (CMN; junctional, compound, dermal, dysplastic naevi), malignant melanoma [superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM)], metastatic melanomas; (4) lung tumour (squamous cell carinoma); (5) mammary gland carcinoma (ductal and lobular from human and rat); (6) nephroblastoma (Wilm's tumours of primary and secondary origin); (7) neurogenic tumours, including retinoblastoma, neuroblastoma, glioma; (8) hypophysical adenomas, including acromegaly, prolactin-producing hypophysial adenoma (prolactinoma) and hormone inactive tumours; (9) paediatric tumours, including Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma, leiomyosarcoma; (10) colorectal carcinomas, consisting of tumour from the rectum, colon, transverse colon, ascending colon, descending colon, sigmoid and rectum-sigmoid; the pathology of these tumours varied from moderately, moderately well, to well differentiated adenocarcinomas consisting of different stages ranging from Stage B to Duke C and with or without lymph node involvement; (11) prostate tumours, including prostate hyperplasia, prostate carcinoma, Dunning R 3327 prostate carcinoma of the rat; and (12) gynaecological tumours, comprising mucinous (including moderately and well differentiated) cystadenocarcinomas, serous (including poorly, moderately, moderately to partly differentiated, moderately well, and well differentiated) cystadenocarcinomas, mixed serous mucinous (i.e. borderline malignancy and well differentiated) cystadenocarcinomas, endometrial (including poorly differentiated, papillary, moderately differentiated, moderately well differentiated, and well differentiated) carcinomas, clear cell (including solid, moderately differentiated, and well differentiated) carcinomas, poorly differentiated cystadenocarcinoma, papillary serous (including poorly and moderately differentiated) cystadenocarcinomas, benign mucinous and benign serous ovarian cystadenomas. In addition, GH receptor expression in rat prostate Dunning R 3327 carcinoma, Yoshida sarcoma, YB2/0 myeloma tumours (Wistar and DA rats, respectively) and in lymphosarcoma of the dog was also investigated.

Established cell lines

Cultures from established cell lines were maintained in Dulbecco's modified Eagle's (DMEM) supplement with 5% fetal calf serum (FCS) in 5% CO_2. Cells were seeded at 3×10^4 viable cells/ml into six-well plates (Nuncion) containing cover slips and cultured to exponential phase and to confluency. Cells were washed twice in DMEM. Cultured cells were then incubated in serum-free medium for 12 h and fixed with PBS containing 4% paraformaldehyde, pH 7.4, for 10 min at room temperature. Cells were stored, if necessary, for short periods only at 4 °C in 70% ethanol until further

treatment. Cells were permeabilised with PBS containing 0.1% Triton X100 before GH receptor immunohistochemistry. The following normal and tumour cell lines were obtained from the Queensland Institute of Medical Research (Brisbane, Australia): human fetal and adult fibroblasts, rat lung and skin fibroblasts, normal and transformed (KJD) human keratinocytes, MRC-5 and HeLa cells, the ovarian tumour cell lines OVCAR, PEO, JAM, G401, RD-ES, GG, LIM125 and the melanoma cell lines MM96, MM170, MM253. The bladder tumour cell lines T24E and T47d7R and the mammary tumour cell line MCF-7 were obtained from the American Tissue Culture Center. Bone marrow cells and long-term culture human bone marrow-derived stromal cell lines were obtained from H. Gabius (University of Munich). The osteogenic sarcoma cells (UMR clones) were obtained from the Department of Medicine, University of Melbourne. The phenotype of this cell line was checked from time to time by measurement of alkaline phosphatase activity.

Tissue-derived cell cultures

For dissociation of tissues following surgical removal into cell suspensions for subsequent cell cultures, tissues were cut into small (ca 2-mm) slices with a multiple-blade tissue slicer on a nylon block. The dispersed cells and tissue were washed once, then resuspended in RPMI 1640 plus 10% FCS with or without 0.1% collagenase and placed in 50 ml Ham's F-12 medium with 0.25% trypsin (Dakopatt) and 1 mg/ml collagenase (Sigma, Type IV). This suspension was incubated at room temperature with gentle shaking. Before aliquots were taken, disaggregation was assisted by agitation with a Pasteur pipette. Samples treated overnight were incubated in a hypoxic CO_2 incubator (5% CO_2, 5% O_2, 90% N_2) at 37 °C with no shaking. Cells were separated from clumps and debris as follows. The cell and tissue suspension was underlayered with FCS in a centrifuge tube. After 7 min, clumps settled through and single cells remained on or above the FCS. The upper single cell layer was removed to another centrifuge tube, again underlayered with FCS, then centrifuged at 100 *g* for 10 min. Cell debris was left in the upper layer and the pellet of single cells suspended in RPMI 1640 plus 10% FCS. The clumps from the initial settling procedure were further treated by a 30-min digestion with 0.1% DNase in RPMI 1640 plus 10% FCS. Viable cell counts were assessed using trypan blue in haemocytometers and cells were plated in six-well plates containing coverslips at a density of 1×10^5/ml and incubated at 37 °C in 5% CO_2 as above. Cultures were fed after 3 days and GH receptor studies were performed at least 3 days later without further feeding. The following human and rat tissue-derived cell culture lines were investigated for GH receptor expression: Ewing's sarcoma, Wilm's tumour, melanoma, rhabdomyosarcoma, neurofibrosarcoma, retinoblastoma, neuroblastoma, Yoshida rat sarcoma and YB2/0 rat myeloma. The melanoma cell line MM96 was established from the lymph node secondaries, MM170 from secondary melanoma right inguinal lymph nodes and MM253 was established from secondary nodes of the left axilla. The primary tumours were at the extreme end of the spectrum of melanomas seen in Queensland, Australia. They all had a high malignant potential with their elevations, profiles, thickness of height, ulceration, absence of in situ component, deep levels of invasion and high mitotic rates.

Production and characterisation of GH receptor MAbs

Four MAbs (MAbs 1 and 5 of IgG2bK isotype, MAb 7 and MAb 43 of IgG1K isotype) to the rabbit GH receptor were produced by application of hybridoma technology to splenic lymphocytes from mice immunised with recombinant human GH affinity-purified preparation of rabbit liver GH receptors (Barnard et al. 1984). These antibodies recognise independent epitopes on the extracellular portion of the receptor in some species, do not crossreact with the related prolactin receptor or with insulin receptor in the appropriate receptor assays and react specifically with the GH receptor in immunoblots (Leung et al. 1987). MAb 7 recognises a species-specific epitope on the rabbit GH receptor only. MAb 263 (IgG1K isotype) was prepared by immunisation of Balb/c mice with purified rat GH receptors as the antigen. This MAb is directed against the hormone binding site of the receptor and recognises a cross-species determinant with high affinity, also present on the human receptor (Asakawa et al. 1986). Immunoreactivity is abolished in human tissues by preadsorption with recombinant GH of the extracellular domain of the GH receptor (Lincoln et al. 1994; Mertani and Morel 1995). MAb 263 is reactive against the receptor in a number of species with high affinity and does not react with insulin or prolactin receptors in rabbit or rat liver. Under certain conditions, MAb 263 precipitates rat and rabbit GH receptor, although it can also compete for hormone binding to subtypes of the receptor. Competition for human receptor is weak at <25% maximum (Werther et al. 1993). For histochemical use, the MAbs were purified from ascitic fluid using: (1) ammonium sulphate precipitation and dialysis; (2) protein-A Sepharose affinity chromatography; or (3) protein-A chromatography followed by ammonium sulphate precipitation and dialysis. Identical distribution of immunoreactivity was seen in all cases, although some loss of intensity was observed with antibody purified using protein-A. This loss was largely overcome by subsequent precipitation with ammonium sulphate and dialysis. Control MAbs (*Brucella abortus* and heartworm 50.6; Agen, Australia) were of the same isotype (IgGK1). The MAbs used in this study have previously been validated immunohistochemically for the recognition of GH receptors in the proliferating mammary gland (Lincoln et al. 1990).

Immunohistochemistry

Serial 5-µm sections were cut from the parafin blocks and collected on polylysine-coated slides. Following deparaffinisation, the presence of GH receptors was demonstrated immunohistochemically by the streptavidin-biotin horseradish peroxidase complex technique. Cultured normal and tumour cells were washed free of culture medium before application of immunohistochemistry. Endogenous peroxidase activity was eliminated by incubation with 0.5% (v/v) hydrogen peroxide in absolute methanol for 15 min at room temperature. Non-specific protein binding was blocked by incubation with 20% normal goat serum in PBS for 1 h at room temperature. Sections were incubated: (1) overnight at 4 °C with primary MAbs against the GH receptor (MAbs 1, 5, 7 at 100 µg/ml, MAb 43 at 50 µg/ml MAb 263 at 12.5 µg/ml, control MAbs *B. abortus* and heartworm MAb 50.6 at 25 µg/ml) in PBS, 1% (w/v) BSA; (2) for 1 h at room temperature with biotinylated goat anti-mouse IgG (Amersham; diluted 1:200 in PBS, 1% BSA); and (3) for 1 h at room temperature with streptavidin horseradish peroxidase complex (Amersham; diluted 1:250 in PBS, 1% BSA). Visualisation was by incubation for 5 min in 0.5 mg/ml diaminobenzidine (DAB) in PBS containing 1% hydrogen peroxide, or with 1 mM 3-amino-9-ethylcarbazole (AEC) containing 0.015% (v/v) hydrogen peroxide in 0.1 M acetate buffer, pH 5.2 for 5–10 min. Between each step, sections were washed 3 times in PBS and once in PBS, 1% BSA. All incubations were carried out in humidified chambers to prevent evaporation. Sections were left non-counterstained or nuclei were lightly counterstained in Mayer's haematoxylin and sections coverslipped with aqueous mounting medium (AEC) or dehydrated, cleared in xylene and mounted with synthetic medium (DAB). Controls were performed by: (1) replacing the anti-GH receptor MAbs with the unrelated heartworm MAb 50.6 or *B. abortus* MAb of the same isotype (IgGK1) at the same concentration; (2) substituting the anti-GH receptor MAbs with normal mouse serum at different concentrations; (3) substituting the anti-GH receptor MAbs 1, 5, 43 and 263 with MAb 7 in rat tissues; (4) omission of the primary monoclonal antibody; and (5) preincubation for 2 h at room temperature with serial dilutions of recombinant GH (Genentech, Calif., USA) at 1, 5, 10, 20 and 30 µg/ml in PBS, 10 mM $MgCl_2$, 1% BSA prior to incubation with primary monoclonal antibody.

Table 1 Expression of growth hormone *GH* receptor immunoreactivity with monoclonal antibody (MAb) 263 in the cell membrane, cytoplasm and nucleus of normal human, rat and rabbit tissues. (+ Labelling of great majority, +(–) labelling of great majority, some negative, +– positive labelling is between 50% and 70%, –+ more than 50% are negative, –(+) more than 90% are negative, – no labelling observed)

Tissue cell type	Localisation of GH receptor immunoreactivity			Tissue cell type	Localisation of GH receptor immunoreactivity		
	Cytoplasm	Nuclei	Species		Cytoplasm	Nuclei	Species
Muscular system				Central nervous system			
Smooth muscle cells	+(–)	–(+)	Human, rat	Hypothalamus neurons	+(–)	+–	Rat, rabbit
Skeletal muscle	+	–(+)	Human rat	Brain stem neurons	+–	+–	Rat, rabbit
Cardiac muscle	+(–)	–(+)	Human, rat	Motor cortex neurons	+–	+(–)	Rat, rabbit
Skeletal system				Reticular neurons	+–	–+	Rat, rabbit
Chondrocytes	+–	–+	Rat, rabbit	Cerebral granular cells	–+	–(+)	Rat, rabbit
Osteoclasts	–(+)	–	Rat, rabbit	Cerebral pyramidal cells	+(–)	+–	Rat, rabbit
Osteocytes	–+	–(+)	Rat, rabbit	Fusiform cells	–+	–(+)	Rat, rabbit
Megakaryocytes	–(+)	–	Rat, rabbit	Neuropil cells	–+	–(+)	Rat, rabbit
Inflammatory cells				Stellate cells	–+	–(+)	Rat, rabbit
Macrophages	+–	–(+)	Human	Hippocampal neurons	+–	–+	Rat, rabbit
Neutrophils	–+	–(+)	Human	Hippocampal pyramidal cells	+(–)	–+	Rat, rabbit
Eosinophils	–(+)	–(+)	Human	Digestive system			
Integument				Epithelial cells	+–	–+	Human, rat
Keratinocytes	–(+)	–(+)	Human, rat	Chief cells	+	–+	Rat
Melanocytes	–+	–(+)	Human, rat	Crypt columnar cells	+	+–	Human, rat
Stratum basale	–(+)	–(+)	Human	Villous columnar cells	+(–)	–+	Human, rat
Hair matric cells	–+	–(+)	Rat	Columnar cells, colon	+(–)	–+	Human, rat
Sebaceous gland cells	–+	–(+)	Human, rat	Columnar cells, caecum	+(–)	–+	Human
Sweat gland cells	+–	–+	Human, rat	Columnar cells, rectum	+(–)	–+	Human
Fibroblasts	+–	–+	Human, rat	Goblet cells	–+	–(+)	Human
Adipocytes	+–	–(+)	Human, rat	Enteroendocrine cells	–+	–(+)	Rat
Vascular				Parietal cells	–+	–(+)	Rat
Endothelial cells	+–	–+	Human, rat	Reproductive system			
Mammary gland				Seminiferous tubule	+–	–+	Rat
Epithelial cells	+(–)	–(+)	Rat, rabbit	Spermatogenic cells	–+	–(+)	Rat
Myoepithelial cells	+–	–(+)	Rat, rabbit	Epididymus, epithelium	+–	–+	Rat
Epithelial cells, pregnant	+	–+	Rat, rabbit	Seminal vesicle, epithelium	+–	–+	Rat
Epithelial cell, lactating	+(–)	–+	Rat, rabbit	Vas deferens, epithelium	+	–+	Rat
Olfactory system				Leydig cells	+(–)	–+	Rat
Epithelial receptor cell	–+	–+	Rat	Sertoli cells	+–	–(+)	Rat
Epithelial support cells	+–	+–	Rat	Prostate gland, epithelium	+	+(–)	Rat
Epithelial basal cells	–(+)	–(+)	Rat	Prostate gland	–	–	Human
Olfactory bulb neuropil	–(+)	–(+)	Rat	Ovary, germinal epithelium	+	+–	Human, rat
Bulb glomeruli cells	–+	–(+)	Rat	Ovary, oocytes	+(–)	+–	Rat
Central nervous system				Granulosa lutein cells	–+	–(+)	Rat
Cerebellar Purkinje cells	+(–)	–+	Rat, rabbit	Corpus luteum cells	+	–(+)	Rat
Cerebellar glial cells	+–	–(+)	Rat, rabbit	Uterus, endometrium	+	–+	Human
Cerebellar stellate cells	–+	–(+)	Rat, rabbit	Uterus, myometrium	+(–)	–(+)	Human
Cerebellar basket cells	–+	–(+)	Rat, rabbit	Endometrial epithelium	+(–)	–+	Human
Cerebellar granule cells	–+	–(+)	Rat, rabbit	Endometrial glands	+	+–	Human
Cerebellar Golgi cells	–+	–(+)	Rat, rabbit	Cervix, uterine	+	–+	Human
Thalamus neurons	+(–)	+–	Rat, rabbit	Cervix, vaginal	+(–)	–(+)	Human
				Vagina	+(–)	–(+)	Human

Results

Reactivity of GH receptor MAbs

Our results immunohistochemically demonstrate the presence of GH binding sites in different tissue cells. Tables 1 and 2 summarise the localisation of GH receptors in the cytoplasm and nucleus of normal and neoplastic tissue cells, respectively, while Table 3 shows cytoplasmic, nuclear and Golgi apparatus expression in cultured cells. Of the five anti-GH receptor MAbs used, only MAb 263 consistently showed immunoreactivity in human tissue cells. MAbs 1, 5, and 7 were completely negative, while MAb 43 resulted in weak staining of some cells in human tissue. The strong immunoreactivity exhibited with MAb 263 is concordant with the specificity of this antibody, which is the only antibody studied experimentally with human tissues. On the other hand, immunoreactivity was present in rat and rabbit tissue cells with MAbs 263, 43, 1, 5 in decreasing intensity of staining, respectively, in the same cell types. MAb 7 was reactive in rabbit tissue cells only, in accordance with its specificity. Subcellular localisation of GH receptor immunoreactivity in the cell membrane, cytoplasm, perinu-

Table 2 Expression of MAb 263 GH receptor immunoreactivity in the cytoplasm and nucleus of neoplastic cells. Proportion of positive cells and nuclei immunoreactivity: –/+ <5%, + 5–15%, ++ 16–30%, +++ >31%

Tumour tissue	Number of cases		Proportion of immunoreactivity	
	Total	Positive	Cytoplasm	Nuclei
Lymphoma				
B-cell lymphoma	9	7	++/+++	+/++
Cutaneous T-cell lymphoma	12	10	+++	–/+/+
Adult T-cell leukaemia/lymphoma	21	18	+++	–/+
Mycosis fungoides	18	15	+++	+
Lymphosarcoma[a]	3	3	++/+++	–/+/++
Basalioma	6	3	–/+/++	–/+/+
Melanoma, benign				
Junctional naevi	12	8	–/+	–/+
Compound naevi[b]	16	9	–/+/+	–/+
Compound naevi[c]	18	12	+	–/+
Dermal naevi	16	10	+/++	–/+
Dysplastic naevi	14	9	+/++	+
Melanoma, malignant				
Lentigo maligna	12	11	++/+++	+
Superficial spreading	15	15	+++	+/++
Nodular	10	10	+++	+/++
Metastatic	13	13	+++	++
Lung tumour				
Squamous cell carcinoma	7	5	+++	+
Mammary Tumour				
Carcinoma, human	36	31	+++	+/++
Carcinoma[d]	15	12	+++	–/+
Nephroblastoma				
Wilm's tumour 1^0	11	9	++/+++	–/+/+
Wilm's tumour 2^0	7	7	+++	+
Neurogenic tumours				
Neuroblastoma	5	3	+++	–/+
Retinoblastoma	3	2	+++	–/+
Glioma	9	7	++/+++	–/+
Hypophysial adenoma				
Acromegaly	5	5	++	+++
Hormone inactive	3	1	+/++	–/+
Prolactinoma	4	3	++	–/+
Paediatric tumours				
Ewing's sarcoma	5	5	+++	++
Osteosarcoma	3	3	+++	–/+/+
Rhabdomyosarcoma	7	7	+++	++
Leiomyosarcoma	5	5	+++	+/++
Colorectal adenocarcinoma				
Rectum	14	12	+++	+/++
Colon	10	9	+++	+/++
Transverse colon	5	5	+++	+/++
Ascending/descending colon	6	6	+++	+/++
Sigmoid colon	5	4	+++	+/++
Rectum-sigmoid colon	6	6	+++	+/++
Prostate tumours				
Hyperplasia	12	0	++	–/+
Carcinoma	18	18	+++	–/+
Dunning carcinoma[d]	6	6	+/+++	–/+
Gynaecological tumours				
Mucin cystadenocarcinoma	12	10	++/+++	+/++
Serous cystadenocarcinoma	31	27	++/+++	+/++
Endometrial carcinoma	27	20	+++	+/++
Clear cell carcinoma	5	4	+++	+/++
Poorly differentiated cystadenocarcinoma	6	4	+/+++	+/++
Papillary serous cystadenocarcinoma	11	9	+/+++	+/++
Benign mucinous ovarian tumour	5	1	–/++	–/+
Benign serous cystadenoma	41	36	–/++	–/+
Yoshida sarcoma[d]	17	17	+++	+
Myeloma YB2/0[d]	25	25	++/+++	–/+

[a] Dog tumour
[b] Epidermal tumour cells
[c] Dermal tumour cells
[d] Rat tumour

Table 3 Expression of MAb 263 GH receptor immunoreactivity in cell cultures of normal and neoplastic cells. Intensity: – negative, + weak, ++ moderate, +++ intense

Cell lines	Reactivity intensity		
	Cytoplasm	Golgi	Nucleus
Epithelial cells			
MRC-5	++	++/+++	+++
HeLa	++/+++	+++	+/++
Fibroblast cells			
Human adult	+	–	–
Human fetal	++	+++	+/++
Rat skin	+	+/++	–/+
Rat lung	++	+	–/+
Bone marrow cells			
Stromal	++	+++	+/++
Haemopoietic	++	++	–/+
Erythroid progenitors	++	++	–/+
Mononuclear leucocytes	+++	++	+/++
Adipocytes	+	–	–/+
Keratinocyte cels			
Normal	+	–	–/+
KJD, transformed	++/++	++	–/++
Ovarian tumour cells			
OVCAR	++	++	+/++
PEO	++	++	+/++
JAM	++	++	–/+
G401	++	++	–/+
RD-ES	++	++	–/+
LIM125	++	++	+/++
GG	++	++	–/++
Melanoma tumour cells			
MM96	++	+++	–/+
MM170	++	+++	–/+
MM253	++	+++	–/+
Bladder tumour cells			
T24E	++/+++	+++	+/++
T47D7R	++/+++	+++	+/++
Mammary tumour cells			
MCF-7	++	++/+++	–/++
Nephroblastoma tumour cells			
Wilm's	++	++/+++	–/+
Paediatric tumour cells			
Osteosarcoma	+++	++/+++	+/++
Rhabdomyosarcoma	++	+++	+/++
Leiomyosarcoma	++	+++	+/++
Ewing's Sch-1	+++	++/+++	+/++
Neurofibrosarcoma	++	++/+++	–/+
Neurogenic tumour cells			
Retinoblastoma	++	++/+++	–/++
Neuroblastoma	++	–/++	–/++
Yoshida sarcoma[a]	++	+/++	–/++
YB2/0 Myeloma[a]	++	–/+++	–/++

[a] Rat cell line

clear Golgi apparatus and nuclei was observed in a variety of cell types from human, rat and rabbit tissue cells. Immunoreactivity was predominantly cytoplasmic, but strong nuclear staining was also apparent in many instances. However, not all cells displaying localisation of the chromogen possessed nuclear GH receptor immunoreactivity.

Ectoderm-derived tissue

Using our panel of MAbs to the GH receptor, we found widespread receptor distribution in brain tissues from neonatal rat and rabbit localised in membrane and cytoplasmic components of neurons and glial cells. Nuclear staining was heterogeneous, sometimes with an open chromatin pattern being more dominant. GH receptor immunoreactivity was present in nerve cells from different regions of the central nervous system, although the proportion of positive cells was varied (Table 1). Prominent levels of immunoreactivity were evident in the cellular cytoplasm, dendrites and nuclei of Purkinje cells (Fig. 1a), the pyramidal cells of the frontal lobe (Fig. 1b), the granular cell layer and fusiform cells of the frontal lobe, the neurons of layer 6 in the neocortex (Fig. 1c), in cells lining the IV ventricle (Fig. 1d), in the nuclei of motor cortex neurons (Fig. 2a), in the cytoplasm and nuclei of neurons in the hippocampus, the thalamus (Fig. 2b), hypothalamus and the brain stem (Fig. 2c). Strong GH receptor expression was also observed in retinal ganglion cells and bipolar cells of the outer plexiform layer. Immunoreactivity showed nuclear and cytoplasmic localisation of the GH receptor in the apical cytoplasm of the olfactory neurons and in many of their nuclei (Fig. 2d). A distinct cytoplasmic immunostaining is also present during mitosis (Fig. 2e). In the mucosa of adjacent respiratory epithelium, a distinct population of the ciliated columnar epithelial cells and respiratory neurons displayed distinct cytoplasmic and nuclear staining, respectively (Fig. 2f). Olfactory bulb glomeruli and periglomerular cells of the glomerular layer as well as the neuropil of the internal and external plexiform layers also displayed prominent GH receptor immunoreactivity, while mitral cells stained moderately to weakly. The relative proportions of GH receptor immunoreactivity in brain tissues are given in Table 1. Expression of GH receptors in normal human adult epidermis, independent of pathological tissue, was rarely observed. On the other hand,

Fig. 1a–f Growth hormone (GH) receptor/binding protein immunoreactivity in central nervous tissue of the rat. **a** Monoclonal antibody (MAb) 263 immunoreactivity in cerebellar glial and granule cells (*small arrow*) and in the nucleus and cytoplasm (*large arrow*) and dendrites (*open arrow*) of Purkinje cells. Visualisation with diaminobenzidine (DAB). **b** MAb 263 immunoreactivity in the nucleus (*solid arrow*) and cytoplasm (*open arrow*) of pyramidal cells located in the frontal lobe of a 10-day-old rat. Visualisation with DAB. **c** MAb 263 immunoreactivity in the nuclei and cytoplasm from the neurons of layer 6 in the neocortex (*arrows*). Visualisation with DAB. **d** MAb 263 immunoreactivity in the cells lining the IV ventricle (*arrows*). Visualisation with DAB. **e** Specificity of anti-GH receptor MAb immunohistochemistry. Layer 6 of the neocortex immunostained with control MAb *Brucella abortus* and nuclear counterstain with haematoxylin (*arrows*). Note the complete lack of immunoreactivity in the nuclei and cytoplasm which are strongly reactive in **c**. **f** GH receptor MAb specificity in control section immunostained with heartworm MAb 50.6 at the same concentration as MAb 263 used in **d** and nuclei counterstained with haematoxylin (*arrows*). Note the complete lack of immunostaining of the cells lining the IV ventricle in contrast to the intense immunoreactivity present with MAb 263 in **d** ▶

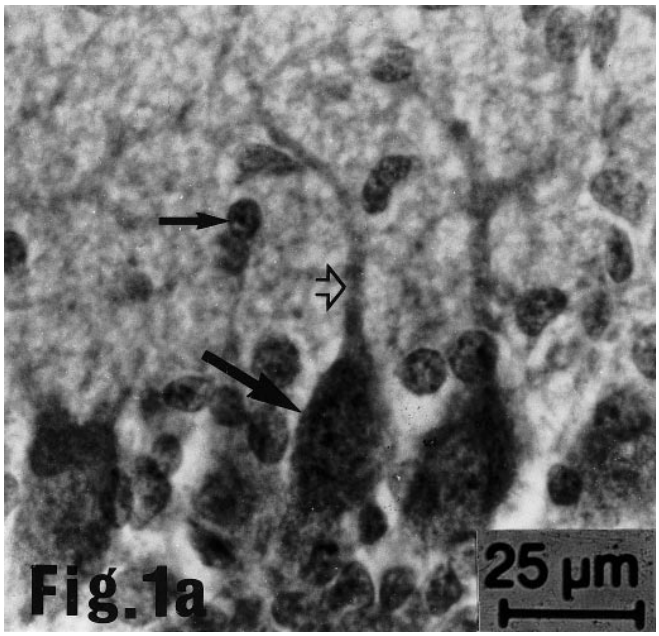

Fig.1a

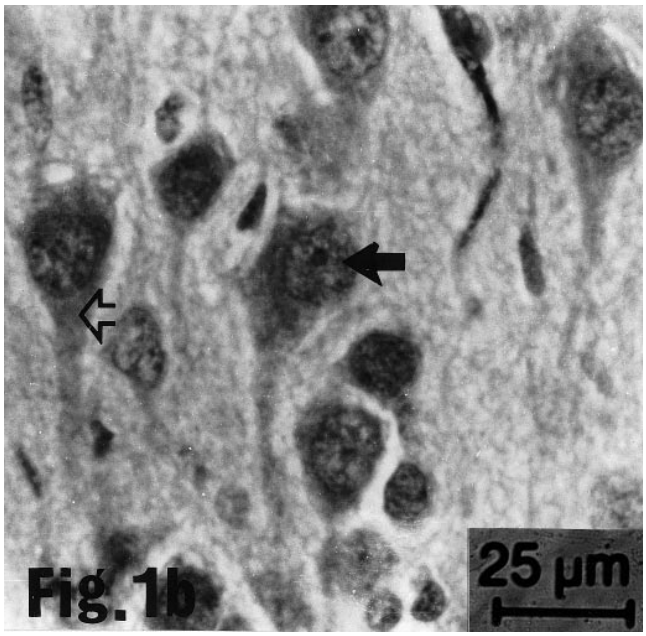

Fig.1b

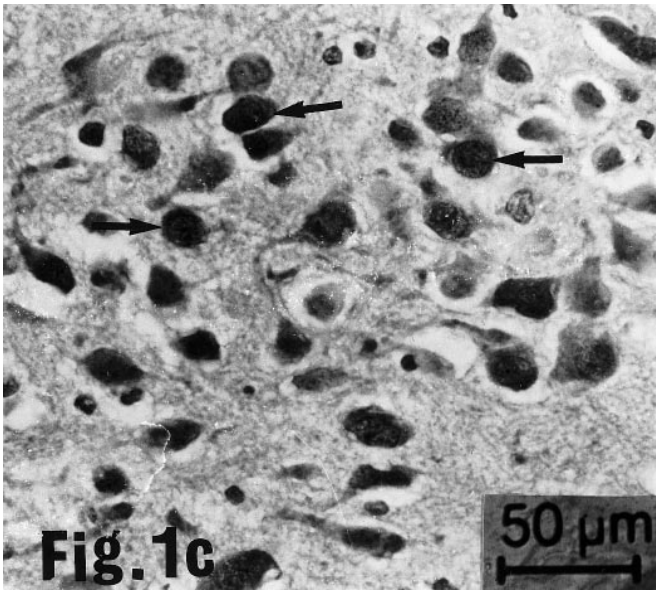

Fig.1c

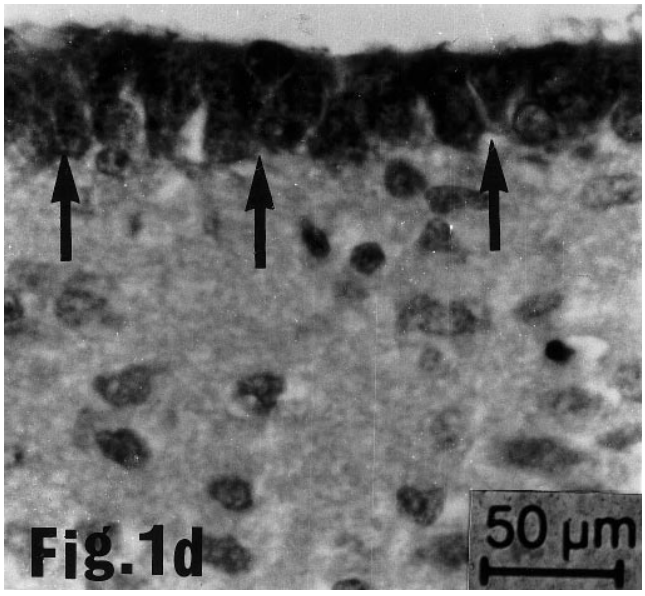

Fig.1d

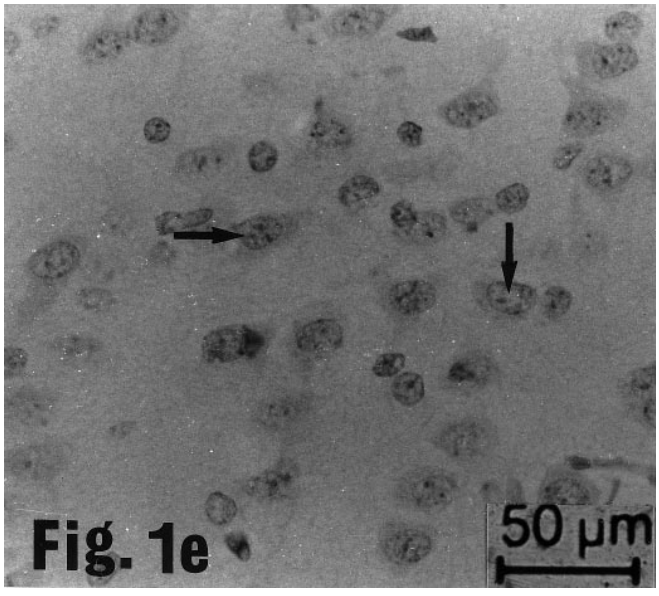

Fig. 1e

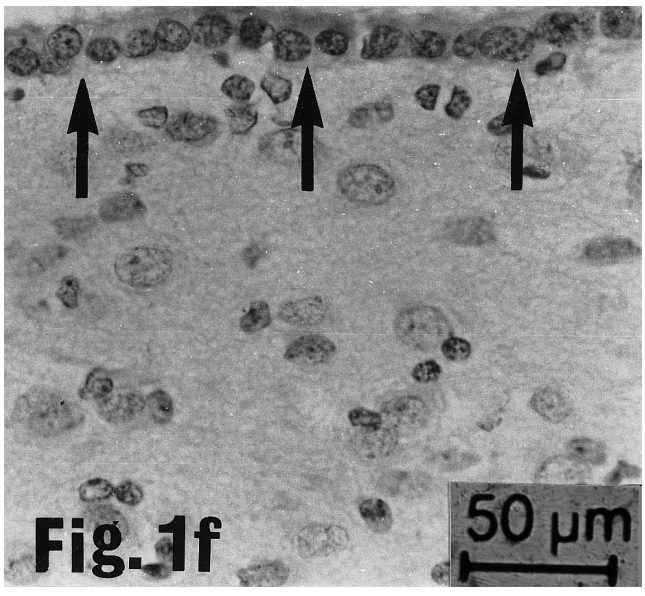

Fig. 1f

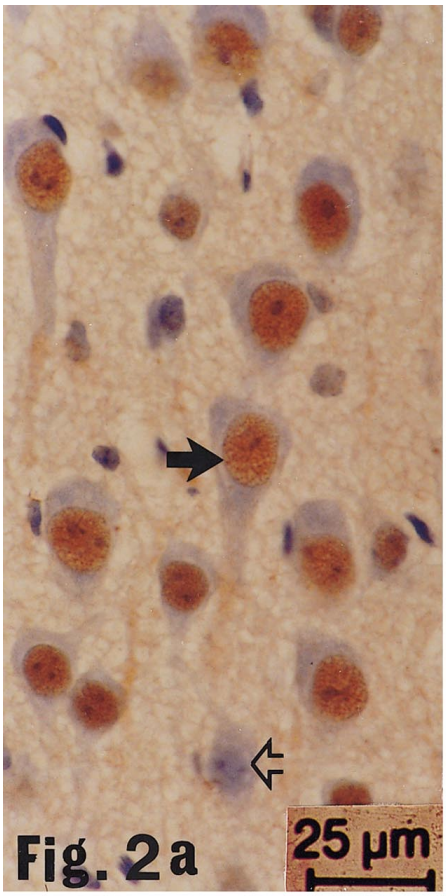
Fig. 2a
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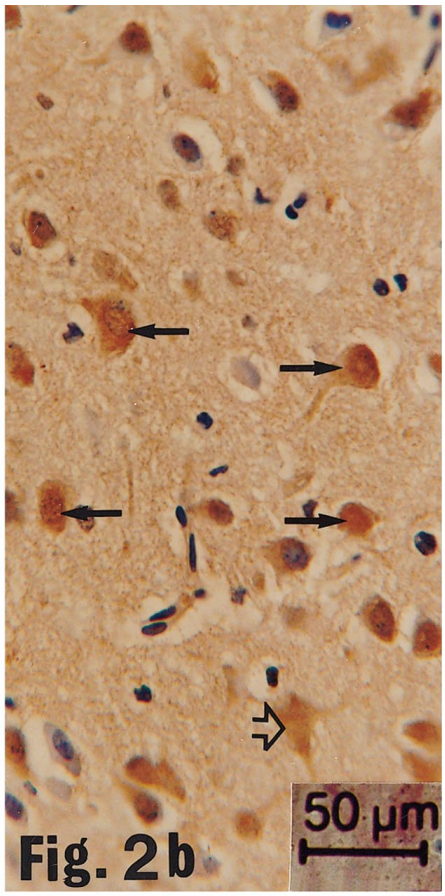
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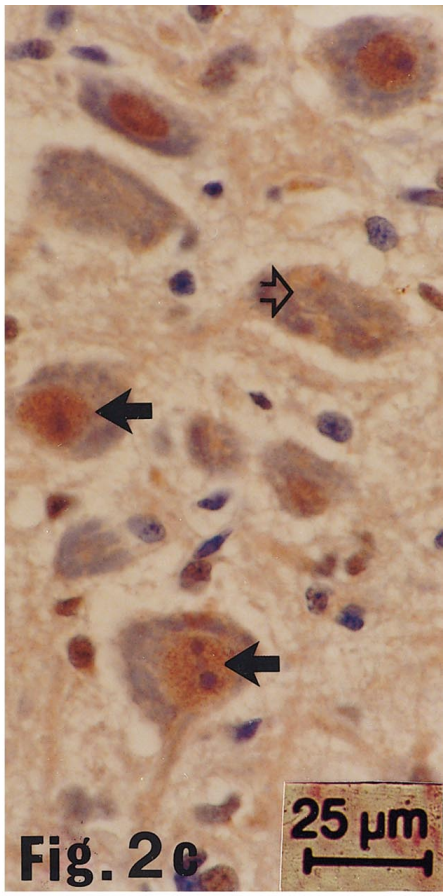
Fig. 2c
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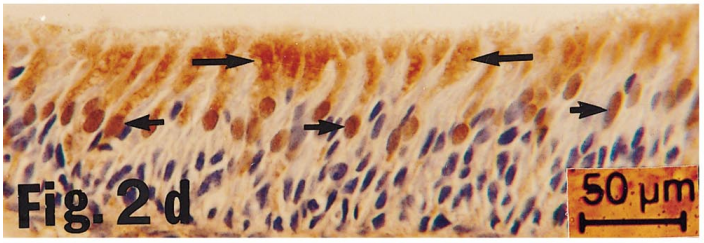
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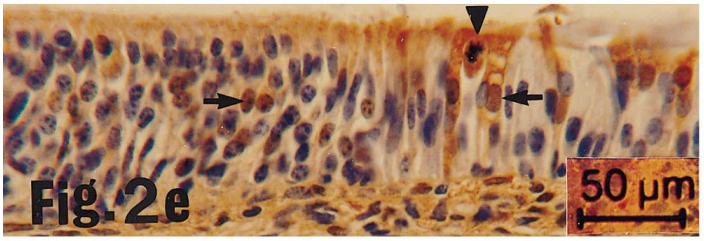
Fig. 2e
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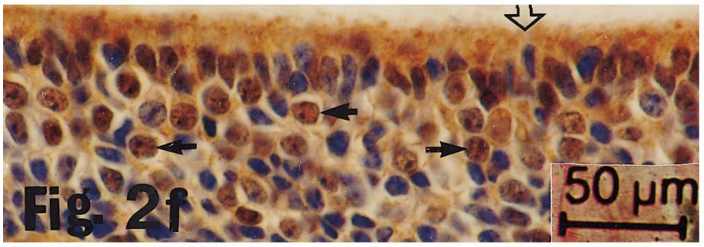
Fig. 2f
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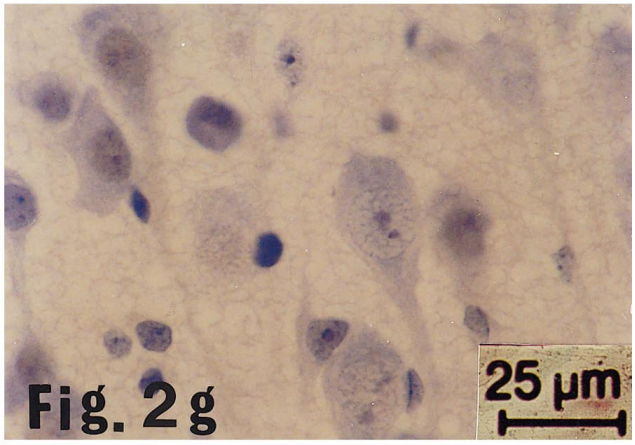
Fig. 2g
25 µm

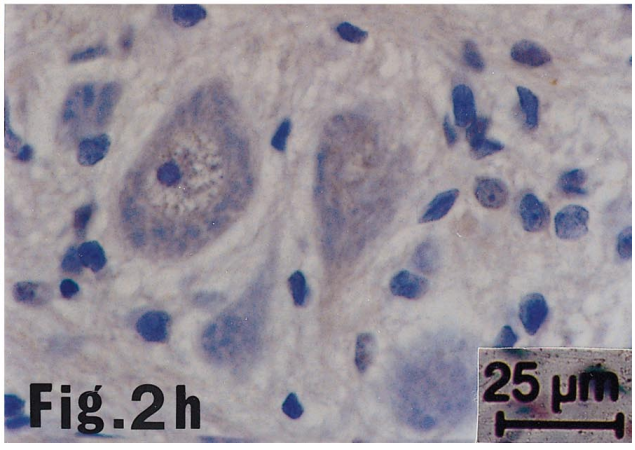
Fig. 2h
25 µm

normal skin, adjacent to or overlaying tumour lesions, showed immunoreactivity in keratinocytes (Fig. 3a), although the intensity and number of positive cells was variable. Basal melanocytes in both normal and pathological tissues were less intensely stained than neoplastic melanoma cells. Metabolically active cells in the dermal appendages of hair follicles, sweat glands and sebaceous glands were strongly immunolabelled in both the nucleus and cytoplasm (Fig. 3b). In the mammary gland, moderate GH receptor expression was present in the proliferating intra- and interlobular and lactiferous sinusoidal epithelial ductal cells, and in tubular glandular and secretory alveolar epithelial cells of pregnant animals. With MAb 263, strong immunoreactivity was observed in the cytoplasm and many nuclei of mammary gland epithelial cells (Fig. 3c) and in myoepithelial cells. During lactation, GH receptor expression was similar in the intra- and interlobular, lactiferous sinusoidal and tubular glandular alveolar epithelial cells, while reactivity in secretory alveolar epithelial cells was moderate. The immunoreactivity intensity of epithelial cells with MAb 263 was reduced during weaning, varying from strong, moderate to weak and thereafter was weak only or negative in the intra- and interlobular and sinusoidal ductal epithelial cells and tubular glandular epithelial cells from regressive mammary glands cells. Immunoreactivity was localised in most of the ductal epithelial cells from both the submandibular and sublingual gland from infant human and rat salivary glands.

Mesoderm-derived tissue

Immunoreactivity of adipocytes, smooth muscle cells, fibroblast and vascular endothelial cells was variable, although a greater proportion of GH receptor-positive fibroblast was evident in the connective tissue sheath surrounding the hair follicle. A distinct nuclear immunoreactivity also occurred in the vascular endothelial cells (Fig. 3d). The smooth muscle cells of the uterus myometrium and uterine cervix were also immunoreactive. Positive staining was seen in a large number of haemopoietic bone marrow precursor cells, mostly of erythroid origin, while some mononuclear leucocytes displayed weak to moderate immunoreactivity. GH receptors were detected immunocytochemically in the nucleus and cytoplasm of a variety of cell types in proliferative and reserve zones of rat growth plate sections (Fig. 3e). Staining was consistently present in chondrocytes from proliferative zones, adjacent to and into the degenerative zone leading to ossification, but only selectively in the nuclei of cells away from proliferative zones (Fig. 3e).

Endoderm-derived tissue

In reproductive tissue, nuclear and cytoplasmic GH receptor localisation was prominent in the simple columnar epithelial cells of the oviduct (fallopian tube), the uterine columnar epithelium, the columnar epithelium of the simple tubular glands in the uterine endometrium, the ovarian germinal epithelium (Fig. 3f), the columnar and squamous epithelium of the endocervical region (squamo–columnar junction), the single layer of tall columnar mucous-secreting cells in the endocervical canal and the stratified non-keratinising squamous epithelium of the vagina. Ovarian primary oocytes and the granulosa lutein cells were variably stained (Fig. 3f). In male reproductive tissue, epithelium of the seminiferous tubules, the pseudostratified epithelium (including its stereocillia) of the epididymis, the pseudostratified cuboidal or columnar epithelium of seminal vesicles, was deferens and the stratified columnar epithelium of the urethra were immunoreactive, although the proportion of positive cells (Table 1) and staining intensity was heterogeneous. Spermatogenic cells in the seminiferous tubules were variably immunoreactive. Compared to spermatids and the mature spermatozoa sex cells, spermatogonia and primary spermatocytes were generally more immunoreactive. The tall columnar Sertoli supporting cells in the testicular epithelium (seminiferous tubules) were homogeneous and moderately immunoreactive. The large ovoid interstitial cells of Leydig were strongly immunoreactive, including some of their eccentrically located nuclei and prominent nucleoli. GH receptor immunoreactivity was not observed in the human adult prostate, either in the glands or in the stroma. In contrast, the rat prostate gland showed strong cytoplasmic and nuclear GH receptor immunoreactivity in epithelial cells during the secretory phase. In normal gastrointestinal tissue, the villous columnar and crypt base columnar epithelial cells from duodenum, jejunum and ileum exhibited strong GH receptor immunoreactivity, as did the crypt base colon columnar and the surface columnar epithelial cells from both the

◀ **Fig. 2a–h** GH receptor/binding protein immunoreactivity in the central nervous system of the rabbit and the olfactory system of the rat. **a** MAb 263 immunoreactivity in the majority of nuclei (*solid arrow*) of neurons located in the motor cortex of a young rabbit. Note absence of immunostaining in some neurons (*open arrow*). Visualisation with DAB. **b** MAb 263 immunoreactivity in the nuclei (*solid arrows*) and cytoplasm (*open arrow*) from the thalamus of a young rabbit. Visualisation with DAB. **c** MAb 263 immunoreactivity in the nuclei (*solid arrows*) and cytoplasm (*open arrow*) in neurons of the brain stem from a young rabbit. Visualisation with DAB. **d** Strong receptor expression with MAb 263 in the cytoplasm of many olfactory neurons (*long arrow*) and many of their nuclei (*short arrow*) from a 10-day-old rat. Note pronounced immunostaining in the apical cytoplasm. Visualisation with DAB. **e** Distinct cytoplasmic immunoreactivity (*triangle*) and nuclei (*arrows*) during mitosis in the olfactory neurons of a 10-day-old rat. Visualisation with DAB. **f** MAb 263 immunoreactivity in the respiratory epithelium of a 10-day-old rat. Note the distinct immunostaining in the population of ciliated columnar epithelial cells (*open arrow*) and nuclei of respiratory neurons (*small arrows*). Visualisation with DAB. **g** Specificity of anti-GH receptor antibody. Neurons located in the motor cortex of a young rabbit pretreated with recombinant GH before application of primary MAb 263. Note the lack of immunostaining in the nuclei, as seen in **a**. **h** Specificity of anti-GH receptor antibody. Neurons located in the brain stem from a young rabbit pretreated with recombinant GH before application of MAb 263. Note the absence of immunoreactivity in the nuclei and cytoplasm in contrast to **c**

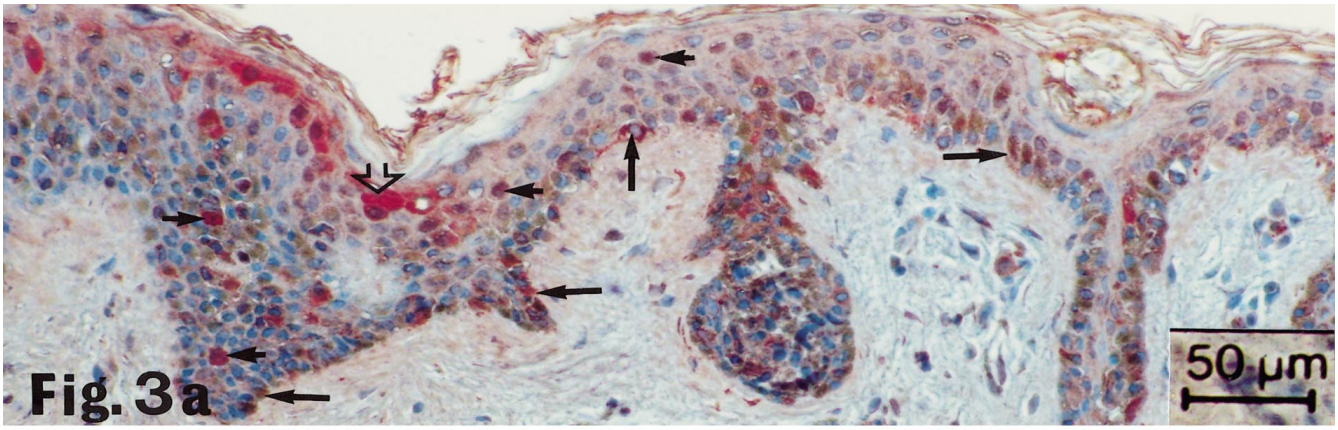
Fig. 3a
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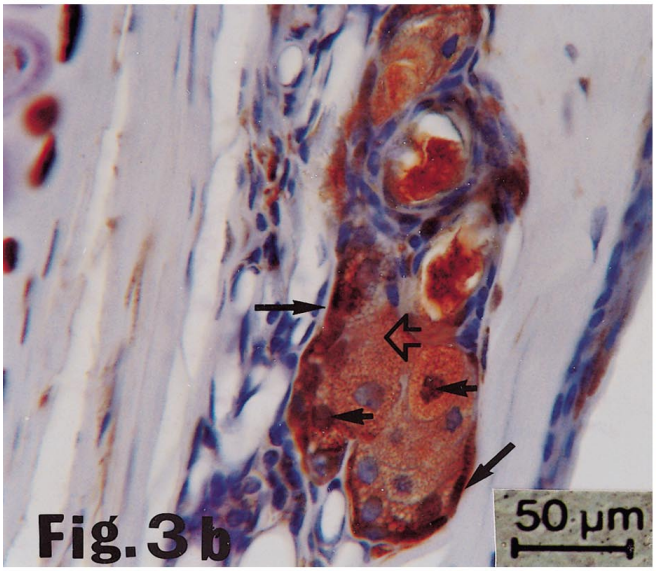
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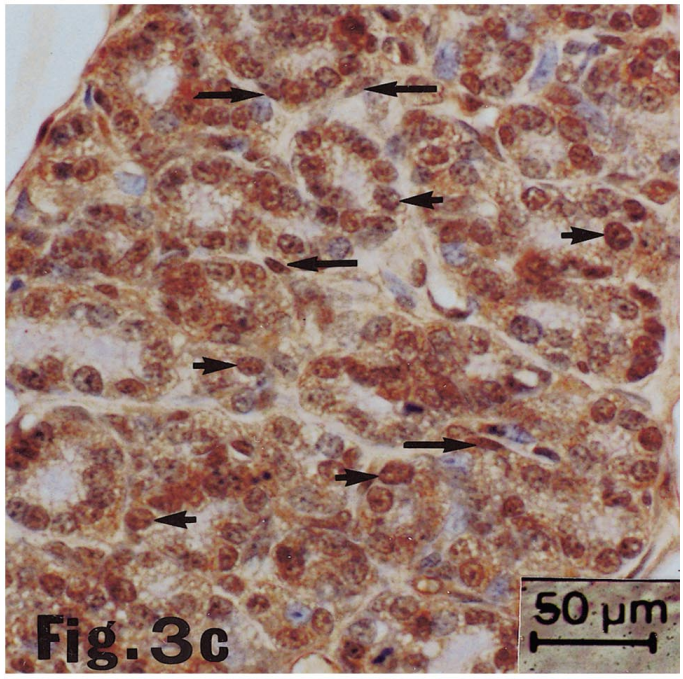
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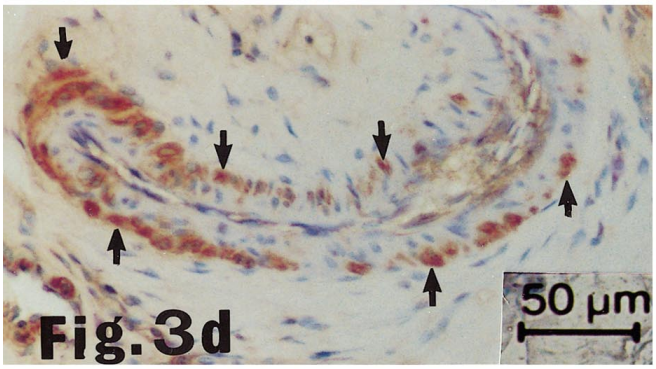
Fig. 3d
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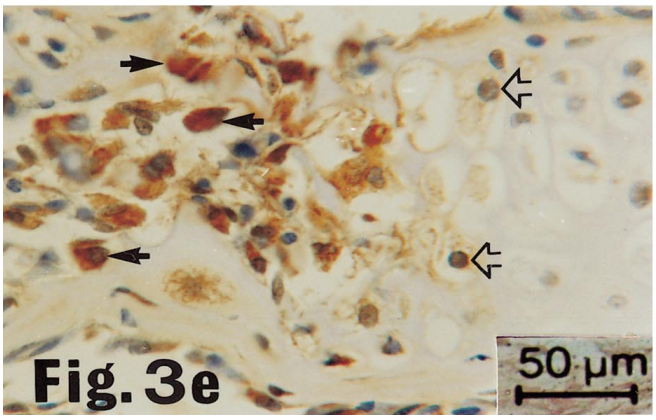
Fig. 3e
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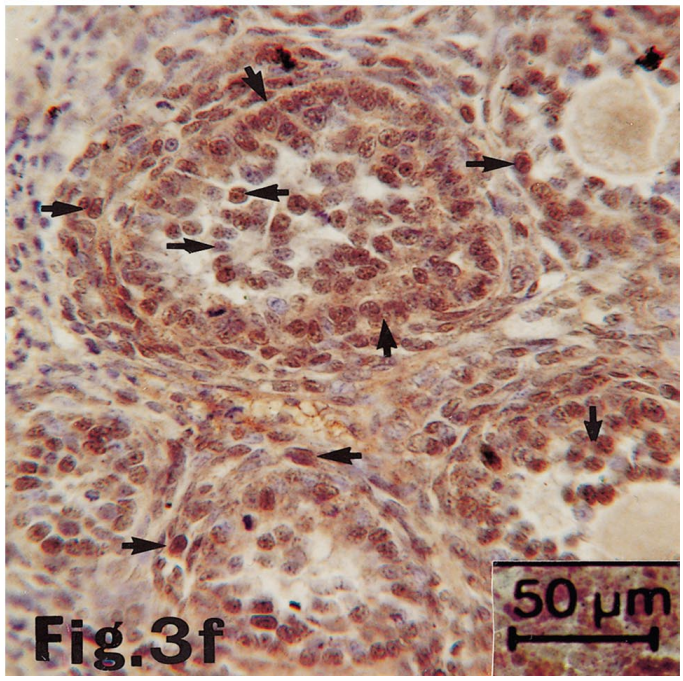
Fig. 3f
50 µm

cecum and colon. Compared to surface columnar cells, the proportion of cytoplasmic and nuclear GH receptor-positive cells was higher in crypt base columnar cells (Table 1), as was the intensity of immunoreactivity. Goblet and enteroendocrine cells displayed considerable heterogeneity of immunoreaction and in the number of cells stained. The proportion of positive parietal cells of the stomach was also variable, with immunoreactivity ranging from generally weak to strong in some cells. Chief (zymogen) cells of the gastric glands displayed intense cytoplasmic immunoreactivity. The number of immunopositive mucous-secreting columnar epithelial cells, located at the stomach fundus and at the base of the pyloric glands, was more than 90% negative (Table 1).

Tumour tissue

Tumour cells from human lymphomas and lymphosarcoma of dog consistently showed intense GH receptor immunoreactivity with MAb 263. The immunoproduct was localised as a homogeneous staining pattern and most of the neoplastic cells were positively labelled (Fig. 4a). Cutaneous T-cell tumours were relatively sharply demarcated as a dense mass of immunoreactive cells of small and medium to large size. In some cutaneous tumours, limited epidermotropism of GH receptor-immunoreactive tumour cells resembled migration of T lymphocytes into the epidermis, as seen in Mycosis fungoides. GH receptor immunoreactivity was present in some nuclei of tumour cells (Table 2). Distinct immunoreactivity with MAb 263 was present in membrane and cytoplasm of the proliferating human ductal and lobular mammary carcinoma tumour cells. Compared to normal mammary gland epithelial cells, GH receptor expression was greatly increased in the spontaneously developed rat mammary gland tumours (Fig. 4b), immunoreactivity varying from moderate to intense depending on the MAb used, paralleling the affinities of the MAbs for the receptor. Epithelial cells of mammary glands adjacent to tumour lesions showed stronger immunoreactivity than epithelial cells of normal proliferating glands. Among the 76 cases of human CMN studied, 48 cases (8 junctional type, 9 epidermal and 12 dermal lesions of compound type, 10 dermal type and 9 dysplastic naevi) were weakly or moderately reactive with MAb 263. In these positive lesions, immunoproduct was mainly localised in the cytoplasm with a homogeneous staining pattern and the proportion of positive cells in lesions was between 5% and 30% (Table 2). Dermal naevus cells localised adjacent to melanoma lesions showed stronger reactivity with MAb 263 than did dermal lesions of ordinary naevi (Fig. 4c), suggesting that expression of GH receptor may be affected through paracrine factors or cell contact phenomena. Table 2 shows that, relative to CMN, the proportion of immunoreactive cells is much greater in malignant melanomas, the intensity ranging from moderately to strongly reactive. Although a considerable heterogeneity of immunoreactivity between each lesion was found in primary melanomas, there was little discernible difference in overall immunoreactivity between SSM, NM and LMM. Immunolabelling by MAb 263 in melanoma cells was mainly observed in the cytoplasm, but membrane and nuclear staining was also seen (Fig. 4d). In primary lesions, dermal tumour cells tended to be more reactive than those seen in the epidermal region. However, there was no significant difference of antigenic expression in tumour cells located in the upper and lower dermis (Clark's level II versus IV). Metastatic melanoma lesions in various organs also demonstrated the presence of GH receptor in tumour cells similar to that seen in primary melanomas, and nearly all tumour cells of the metastatic lesions were positive (Table 2). Immunolabelling with MAb 263 in human gynaecological tumour cells was observed in the cytoplasm with either a monotonous or a granular staining pattern. Immunoreactivity was present also in the nuclei of some tumour cells which also displayed cytoplasmic localisation of the receptor (Fig. 4e). The presence of GH receptor in human colorectal carcinoma tumour cells is shown in Fig. 4f. Epithelial cells from normal tissue, adjacent to colorectal tumour lesions, showed immunoreactivity with variable intensity. Goblet cells immunostained faintly or were negative for GH receptor. Mucous cells located at the crypt base were immunonegative. Crypt base columnar cells were strongly immunoreactive in tumour lesions, but oligomucous cells were less reactive. In contrast to the normal human prostate, GH receptors are present in human prostatic hyperplasia and in human prostatic carcinoma. Immunoreactivity was located throughout the epithelium of the tumour acini. The staining was confined to the cytoplasm. The most intense staining reaction was in the apical areas of the glandular epithelial cells. Immunoreactivity was pro-

◀ **Fig. 3a–f** GH receptor/binding protein immunoreactivity in normal tissues. **a** MAb 263 immunoreactivity in human skin adjacent to malignant melanoma. Note variable receptor expression in the cytoplasm (*open arrow*) and nuclei (*short arrows*) of keratinocytes and in the basal melanocytes (*long arrows*). Visualised with 3-amino-9-ethylcarbazole (AEC). **b** MAb 263 immunoreactivity in the sebaceous gland of a young rat. Note cytoplasmic immunolabelling (*open arrow*) and intense reactivity in some nuclei (*short arrows*) of the sebaceous gland. Myoepithelial cells also express intense immunoreactivity (*long arrows*). Visualised with DAB. **c** MAb 263 immunoreactivity in the mammary gland from a 16-day pregnant rat. Note strong immunolabelling in the cytoplasm and many nuclei (*short arrows*) and in the myoepithelial cells located at the periphery of the tubular glandular and secretory alveolar epithelial cells (*long arrows*). Visualised with DAB. **d** MAb 43 immunoreactivity in vascular endothelial cells from a 20-day-old rat. A distinct nuclear receptor expression is also present in many cells (*arrows*). Visualised with DAB. **e** MAb 43 immunoreactivity in the nucleus and cytoplasm of cells from the growth plate zone from the long bone of a 10-day-old rat. Note consistent staining of chondrocytes from the proliferative zone (*solid arrows*) but only selective reactivity in the nuclei of some cells away from the proliferative zone (*open arrows*). Visualised with DAB. **f** MAb 263 cytoplasmic and intense nuclear immunoreactivity (*arrows*) in the ovarian germinal epithelium and in the granulosa and theca cells from the ovary of a rat. Visualised with DAB

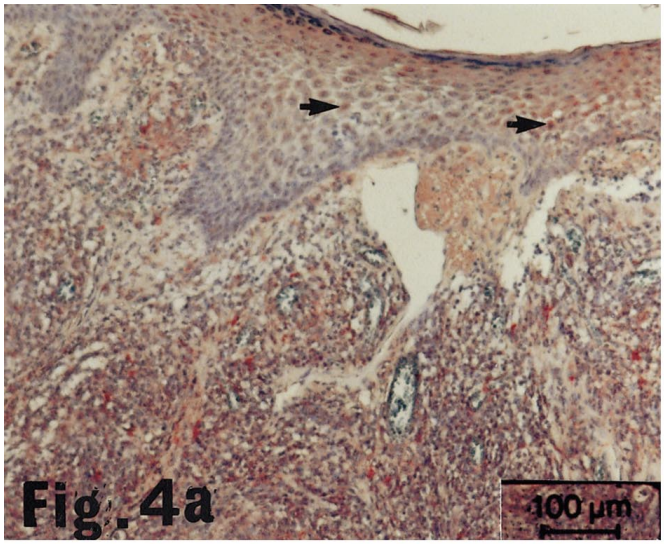
Fig. 4a
100 µm

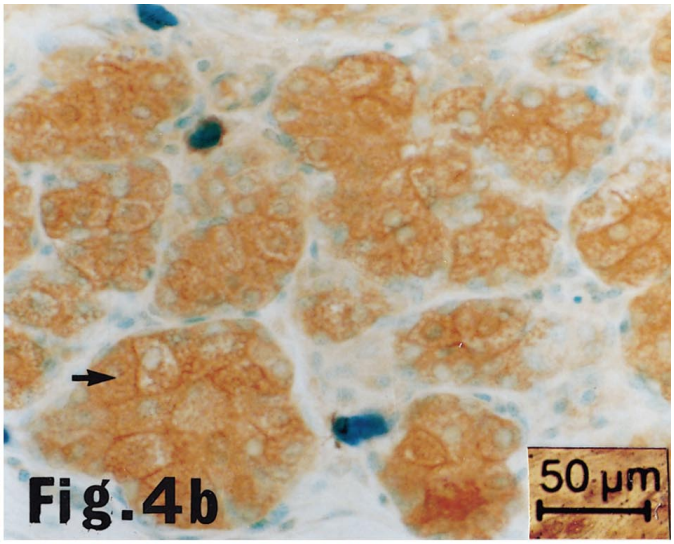
Fig. 4b
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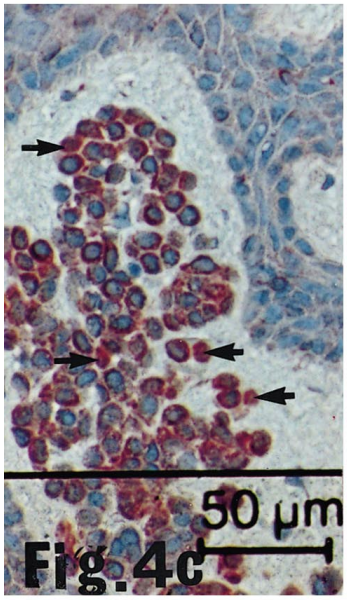
Fig. 4c
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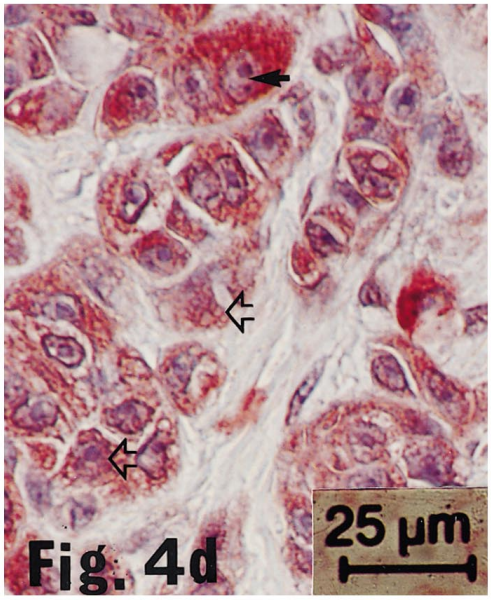
Fig. 4d
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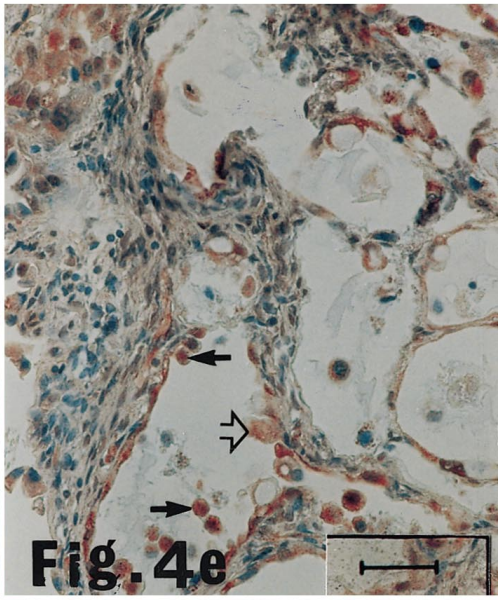
Fig. 4e

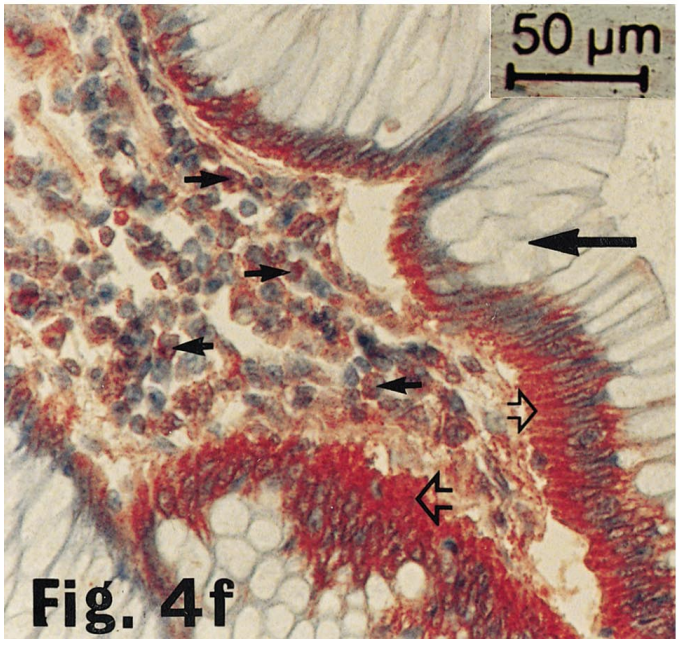
Fig. 4f
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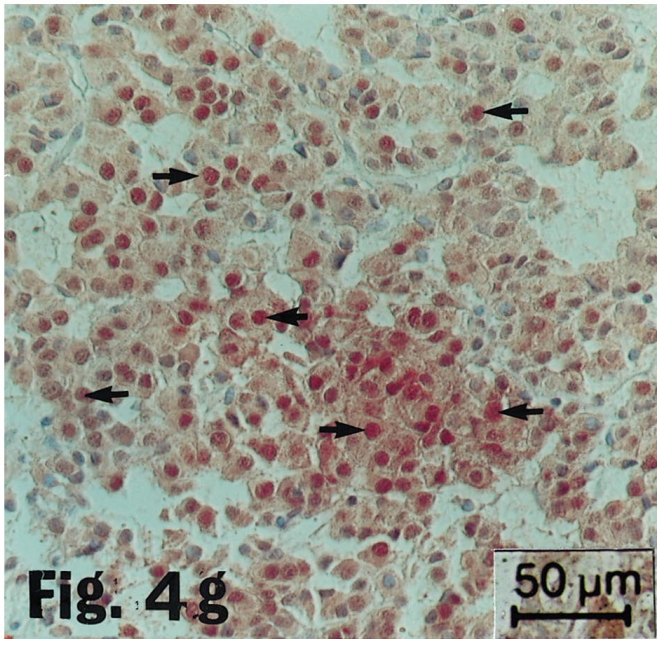
Fig. 4g
50 µm

nounced in the epithelial cells in prostatic carcinomas. Altogether, staining of the epithelial cells in prostatic carcinomas was somewhat more intense than that in prostatic hyperplasia. In the rat, weak GH receptor immunoreactivity was present throughout the epithelium of the tumour acini from the Dunning R 3327 prostatic rat carcinoma. Large epithelial cells, located at the periphery of the acini, displayed an especially strong binding of MAb 263. GH receptor immunoreactivity was always confined to the cytoplasm. Nuclear staining was not seen. Staining was weak in the multilayered epithelium of the large ducts. Large neoplastic epithelial cells in the stroma had pronounced GH receptor immunoreactivity. Of the 12 cases of hypophysial adenomas investigated, 9 cases showed positive immunoreactivity (Table 2). Nuclear immunoreactivity was most pronounced in all cases with acromegaly, with a high proportion of positive staining (Fig. 4g).

Cultured cells

Localisation of GH binding sites was prominent in cell membrane, cytoplasm, extranuclear Golgi area and nuclei of the cell lines investigated (Table 3). Reactivity intensity was most pronounced over the Golgi area in exponential growth phase cells, but was weak to negative in cultures of stationary confluency. Prominent nuclear GH receptor expression was observed in MRC-5 cells (Fig. 5a). Immunostaining was more intense in fetal human fibroblast cells than in adult fibroblasts. In cultures of bone marrow cells, positive staining was seen in a large number of haemopoietic bone marrow precursor cells, mostly of erythroid origin, while some mononuclear leucocytes displayed weak to moderate immunoreactivity. Receptor expression was present in small proliferating progenitor cells, large reticular fibroblasts, adipocytes and endothelial cells and was prominent in the Golgi apparatus of adherent fibroblastoid cells of all donor cell lines investigated (Fig. 5b). Compared to normal keratinocytes, immunoreactivity was more intense in transformed KJD cells, while receptor expression was intense over the Golgi area in melanoma cells (Fig. 5c). Expression of MAb 263 GH receptor immunoreactivity was also prominent in all tumour cell lines investigated, being most pronounced over the Golgi area and present in the nuclei of cells from different cell lines, including human ovarian tumour cells (Fig. 5d) and rat Yoshida sarcoma cells (Fig. 5e).

Specificity

Both MAb 43 and MAb263 were immunoreactive in identical locations, even though they recognise independent epitopes on the GH receptor/binding protein. Immunostaining was observed at the same locations with MAb 1, although this was of lower intensity in accord with its lower affinity for the receptor. MAb 263 is also reactive against human tissue and the location of immunoreactivity was identical to that seen in other species. Immunoreactivity with MAb 7 was present in rabbit tissue only. MAb 7 is non-reactive with the rat receptor and no staining could be obtained, although sections were strongly reactive with three other anti-GH receptor MAbs (263, 43 and 1). The intensity of GH receptor localisation, but not the location, was found to be dependent on the antibody preparation technique. For MAb 263, the most intense immunoreaction was observed with ascitic fluid precipitated with ammonium sulphate and dialysed. Protein-A purification resulted in a decrease in immunoreactivity, a degree of which was restored by ammonium sulphate precipitation and dialysis subsequent to protein-A purification. Use of protein-A-purified and protein-A/ammonium sulphate-precipitated antibody at a four-fold concentration (5 mg/ml; 1:dilution) resulted in comparable staining to ascitic fluid/ammonium sulphate-precipitated antibody. Cell cultures or tissue sections incubated with 20% normal goat serum, without the primary monoclonal antibody or with unrelated primary antibodies of identical isotype (*B. abortus*, IgG1K; heartworm MAb 50.6, IgG1K) at the same concentration as the test MAbs, did not display detectable immunoreactivity (Fig. 1e, f). Incubation with MAb 7 did display immunoreactivity in rabbit tissues, but not in rat tissues. MAb 7 is not reactive with the rat GH receptor. Pretreatment of cell cultures/tissue sections with recombinant human GH before application of the primary antibody (MAb 263) abolished staining both in the nucleus and the cytoplasm (Fig. 2f, g). On serial dilutions of growth hormone at 1, 5, 10, 20 and 30 µg/ml, graded levels of immunoreactivity were observed. Immunoreactivity was reduced after preabsorption at 5 µg/ml followed by incubation with MAb 263 and was absent with preincubation at 20–30 µg/ml. GH receptor immunoreactivity was more prominent in cells/sections when preincubated with 5 µg/ml re-

◀ **Fig. 4a–g** GH receptor/binding protein immunoreactivity in neoplastic tissue cells. **a** MAb 263 immunoreactivity in a highly malignant human Mycosis fungoides T-cell lymphoma. Note most of the neoplastic cells are positively labelled and migration of T lymphocytes into the epidermis (*arrows*). Visualisation with AEC. **b** MAb 43 immunoreactivity in tumour cells (*arrow*) from a spontaneously developed rat mammary gland carcinoma. Visualisation with DAB. **c** MAb 263 immunoreactivity in the cytoplasm and some nuclei (*arrows*) of human dermal naevi cells. Visualisation with DAB. **d** MAb 263 immunoreactivity in the nuclei (*solid arrow*) and cytoplasm (*open arrows*) of tumour cells from a highly malignant human melanoma located in the deep dermis. Visualisation with AEC. **e** MAb 263 immunoreactivity in the cytoplasm (*open arrow*) and some nuclei (*solid arrows*) of a human clear cell carcinoma of the ovary. Visualisation with AEC. *Magnification bar* 50 µm. **f** MAb 263 immunoreactivity in tumour cells (*small arrows*) from a human colorectal carcinoma. Note intense immunolabelling in the epithelial cells (*open arrows*) of the tumour and absence of receptor expression in the goblet cells (*large arrow*). Visualisation with AEC. **g** MAb 263 immunoreactivity in the cytoplasm and nuclei of tumour cells of a hypophysical adenoma with acromegaly. Note pronounced nuclear receptor expression (*arrows*) with a high porportion of positive staining. Visualisation with AEC

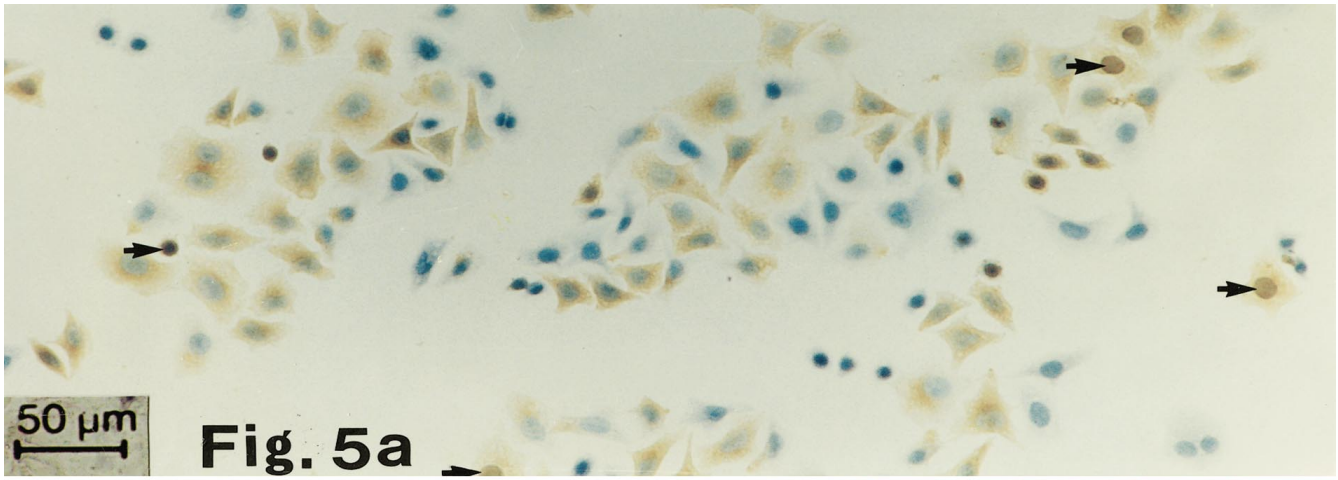

Fig. 5a

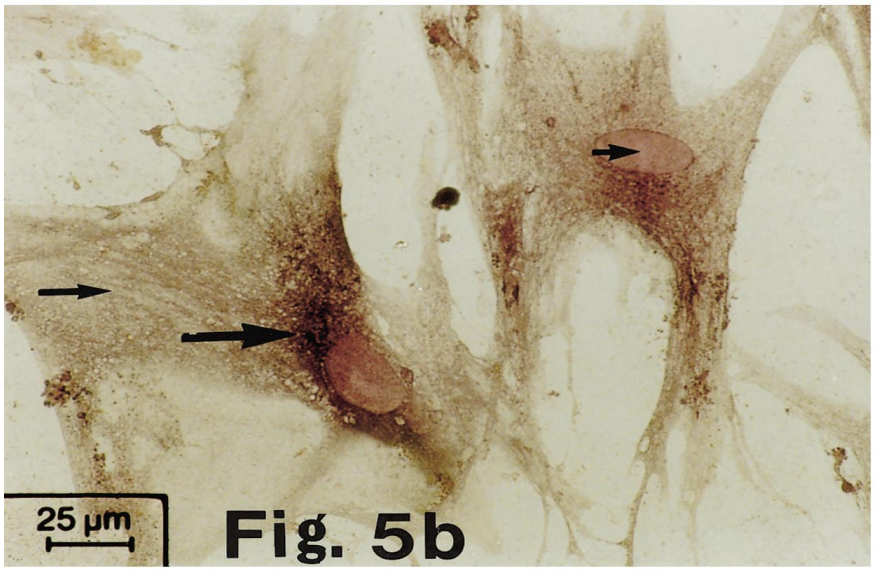

Fig. 5b

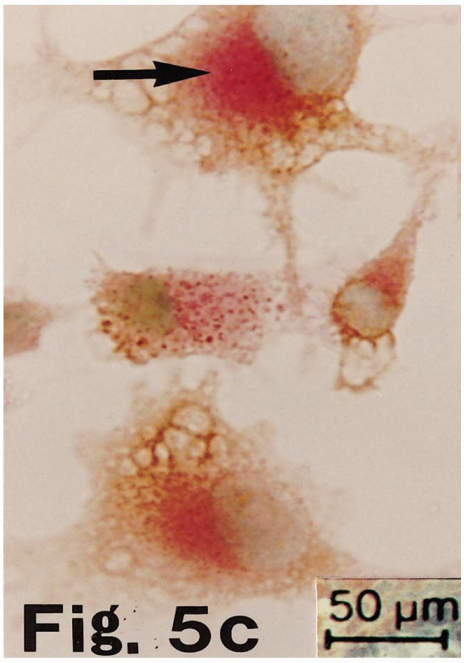

Fig. 5c

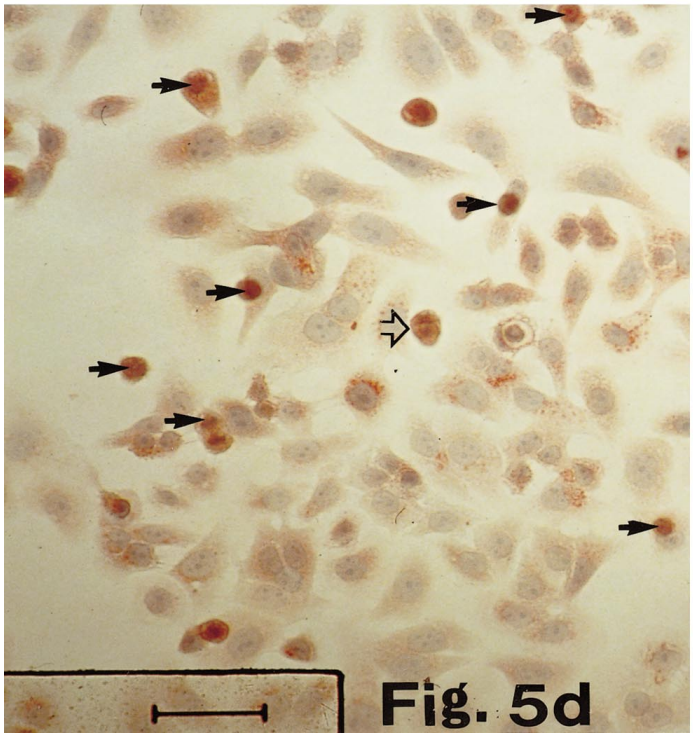

Fig. 5d

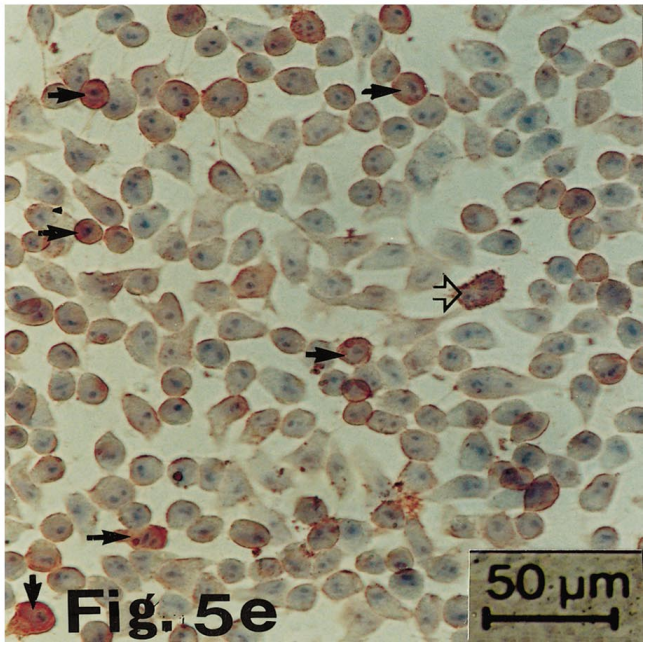

Fig. 5e

combinant human GH followed by MAb 263, instead of the control MAbs from *B. abortus* or heartworm.

Discussion

Cytoplasmic localisations of GH receptors have been reported in cells such as lymphocytes (Lesniak and Roth 1976), fibroblasts (Murphy and Lazarus 1984) and macrophages (Kover et al. 1986) by convential radio-ligand binding studies and, more recently, by using MAbs against the extracellular portion of the GH receptor in rat prostatic epithelial cells (Sinowatz et al. 1991), human bone marrow cells (Lincoln et al. 1992), rat cerebellum (Lincoln et al. 1994), primary Ki-1 lymphoma of human skin (Lincoln et al. 1995a) and in human stromal subpopulation progenitor cells (Lincoln et al. 1997). Our results from this study demonstrate the subcellular localisation of GH receptor/binding protein in the cell membrane, cytoplasm and perinuclear Golgi apparatus, as well as at times to the nucleus of normal and neoplastic cells, implying that GH acts directly on these cells at the gene level and its action may be mediated without hepatic production of IGF-I, as claimed by the somatomedin hypothesis. The expression of GH receptors not only on small proliferating tumour cells, but also on well differentiated cells, suggests that GH is also necessary for, or has a trophic function in, differentiation. We propose that direct GH action is necessary not only for differentiation of progenitor cells, as implied by the dual effector hypothesis (Green et al. 1985), but also for their subsequent clonal expansion, differentiation and maintenance. The detection of GH receptors in cultured cells is of particular interest. Although it is difficult to identify the location of the immunoreaction product in the Golgi area under the light microscope in tissue sections, the subcellular localisation of GH receptor in this area was amply confirmed in cultured cells. The significance of GH receptor expression in the Golgi area is not clear, but this compartment has a high level of GH receptor expression in hepatocytes (Husman et al. 1988) and presumably represents synthetic events. Immunoreactivity over the Golgi region indicates the cells are capable of GH receptor synthesis. Previous studies on transformed human KJD keratinocyte cells indicated that GH receptor expression correlates with nuclear proliferating cell nuclear antigen/cyclin proliferation (Lincoln and El-Hifnawi 1994). The presence of GH receptors suggests that GH is the likely driving force in maintaining receptor expression and acts directly on these target cells to trigger growth at the cellular level. Whether the receptor is required only for internalisation and not intracellular movement of the hormone is not known. It is possible that, once internalised, the hormone–receptor complex could dissociate and the hormone be translocated by a receptor-independent pathway, perhaps complexed to the GHBP, the biological function of which is not fully understood (Herington et al. 1991).Our findings provide evidence that GH receptor expression in epithelial cells associates with mammary gland proliferation during pregnancy and lactation, and suggests a direct effect of GH on these cells during development and lactation. Receptor expression in stromal cells correlates with the distribution of IGF-I mRNA from in situ hybridisation immunohistochemistry in stromal breast tissue (Yee et al. 1989). Whether growth-promoting activities of GH on mammary gland tissue results in production of IGF-I, which would promote growth by a paracrine or autocrine mechanism, is unknown. IGF-I expression is prominent in the cytoplasm of mammary epithelial cells after somatotropin treatment (Glimm et al. 1988). The existence of GH receptors in the mammary gland implies the availability of GH or its variants to trigger these receptors and does explain the strong galactopoietic effect of GH on milk production. The ductular epithelium of the gestational mammary gland shows intense immunoreactivity in the period of rapid growth during gestation and in lactation, implying a role for GH in duct formation, as was demonstrated many years ago ly Lyons (1958) and, more recently, with local implants of GH (Silberstein and Daniel 1987). Intensity is less in the secretory alveolar epithelial cells during lactation, is further reduced in all non-secretory epithelial cells during weaning and is absent or only weak in cells of regressive mammary glands. Such a process would enhance tissue sensitivity to circulating GH during the rapid growth period (Lincoln et al. 1995b). Our finding of GH receptors in mammary tissue challenges the view that GH has no direct influence on mammary growth or function and is further supported by evidence for the presence of GH receptor mRNA in lactating bovine gland tissue by northern and in situ hybridisation analysis (Glimm et al. 1990). Results from this investigation also demonstrate a wide distribution of GH receptor immunoreactivity in the digestive tract epithelial cells. This is not surprising since GH has several distinct roles in intestinal physiology, including increased mitotic activity of the duodenal epithelium and the crypts of Liberkühn (Leblond and Carriere 1955). Hypertrophy of the intestinal tract to supranormal levels following administration of GH to hypophysectomised rats has been reported (Scow and Hagan 1965). Both GH and insulin are neces-

◀ **Fig. 5a–e** GH receptor/binding protein immunoreactivity in cultured cells. **a** MAb 263 immunoreactivity in MRC-5 cells. Note prominent nuclear receptor expression (*arrows*) in some cells. Visualisation with DAB. **b** MAb 263 immunoreactivity in the nucleus (*small arrow*) and cell membrane (*medium arrow*) of stromal adherent fibroblastoid cells derived from cultured human bone marrow cells. Note intense receptor expression in the perinuclear Golge apparatus (*large arrow*). Visualisation with DAB. **c** MAb 263 immunoreactivity in human MM96 melanoma cells derived from metastasised lymph node cells of a highly malignant melanoma. Note intense receptor expression over the Golge area (*arrow*). Visualisation with AEC. **d** MAb 263 immunoreactivity in human G 401 ovarian tumour cells. Note intense receptor expression in some nuclei (*arrows*) and in mitosis (*open arrow*). Visualisation with AEC. *Magnification bar* 50 μm. **e** MAb 263 immunoreactivity in rat Yoshida sarcoma cells. Note prominent nuclear receptor expression (*arrows*) and in mitosis (*open arrow*) in some cells. Visualisation with AEC

sary for normal growth of transplanted rat intestine during late fetal and early neonate life, but only GH is required for normal differentiation (Cooke et al. 1986). High levels of GH receptor mRNA have also been reported in the rat intestine (Mathews et al. 1989). The expression of GH receptors in the central nervous system is intriguing. Because pituitary removal has no effect on brain growth, and since GH does not pass the blood–brain barrier, brain growth and function are regarded as being independent of GH. Immunohistochemical detection of the receptor correlates with the demonstration of receptor mRNA expression in the same locations by in situ hybridisation (Lobie et al. 1993). Receptor location corresponds to areas of IGF-I expression in published studies, the mitogen being the intermediate in the somatic actions of GH and increases in the brain in response to intracerebroventricular GH administration (Hynes et al. 1987). Since it is known that GH is produced within the brain (Gossard et al. 1987), we can postulate a brain GH axis regulating its growth and development. The reported retardation of visuomotor and intellectual function in Laron dwarfs (Frankel and Laron 1968), who display a specific GH receptor defect, provides clinical support for this hypothesis.

Distinct GH receptor immunoreactivity was present in human and rat mammary carcinoma and cultured MCF-7 tumour cells. Localisation of the receptor in proliferating mammary carcinoma cells suggests that the tumour may be stimulated by GH and raises questions regarding the possible oncogenic potential of the GH receptor. Receptor expression may be associated with malignant transformation of the mammary gland. Furthermore, the mitogenic intermediate in GH action, IGF-I, has been shown to be overexpressed in breast carcinoma (Yee et al. 1989) and IGF-I binding was higher in mammary gland tumour tissue than in adjacent normal tissue (Artega and Osborne 1989). This implies that increased IGF-I may be associated with malignant transformation of breast mammary epithelial cells. Cultured human breast cancer cell lines also produce and secrete IGF-I (Huff et al. 1986). In this regard, Conte and Gardin (1990) found that recombinant human GH largely enhances human breast tumour proliferation. Furthermore, the presence of GH receptors in colorectal carcinoma and ovarian tumour cells provides evidence that GH also acts directly on these tumour cells and our results partially support the modified somatomedin or dual effector hypothesis of GH action, which proposes that both GH and IGF-I act to promote growth, GH being required for differentiation of progenitor cells and IGF-I stimulating their subsequent clonal expansion (Isaksson et al. 1987). IGF-I has been shown to be overexpressed in colon carcinomas (Tricoli et al. 1986). In addition, several clinical surveys (Pines et al. 1985; Brunner et al. 1990) and case reports (Ziel and Peters 1988) have suggested an increased incidence of colon cancer in patients with acromegaly, including adenomatous colonic polyps and carcinoma of the colon. Ron et al. (1991) also reported an increased rate of cancers of the digestive organs, including colon cancer. Our results also show a positive immunoreaction in lymphoma tumour cells. This is in agreement with published identification of GH receptor in cultured human lymphocytes (IM 9 cell line) by use of radio-ligand binding techniques (Hughes and Friesen 1985). Both GH and IGF-I are reportedly involved in the regulation of the immune system. It is not clear, however, whether GH directly regulates lymphocyte function via its own receptor (Lesniak et al. 1985), or indirectly via IGF-I and its receptor on lymphocytes (Kozak et al. 1987). The increase in GH receptor immunoreactivity observed between melanocytes of control skin, naevi, primary melanomas and metastatic melanoma implies that receptor expression is a function of tumour progression. The heterogeneous staining of normal melanocytes in pathological specimens may result from a paracrine effect in these lesions. Functional naevus cells are believed to proliferate and then become quiescent when they move down into the dermis (Paul and Gerhardt 1983). Our results of GH receptors in human prostatic hyperplasia and carcinoma supplement the findings of Sibley et al. (1984), who detected endogenous human GH in tumour tissue from patients with benign prostatic hyperplasia or prostatic carcinoma. The role of GH in prostatic hyperplasia and carcinoma was first investigated by El Etreby and Mahrous (1979). They indirectly confirmed the existence of GH binding sites in hyperplastic and neoplastic lesions of dog prostates by preincubation of canine prostatic cancer cells with ovine GH and detection of GH by immunohistochemistry. Receptors for GH have also been demonstrated in the Dunning R 3327 prostatic carcinoma of the rat (Sinowatz et al. 1991), which showed strong GH receptor immunoreactivity in large cells located in the periphery of the acini and in large cells in the stroma. In this study, cytoplasmic and nuclear GH receptor immunoreactivity was pronounced in hypophysical adenomas, particularly in all cases with positive acromegaly. According to some investigators, the incidence of neoplasm in acromegaly is increased (Bengtsson et al. 1988).

Changes in the level of growth factors/receptors may be important in the pathogenesis of a number of different types of tumours. Elevated plasma levels of GH have been documented in patients with numerous malignancies (Adamson et al. 1980; Andrew 1983), but the effect of GH in tumour pathogenesis is unknown. The administration of GH also significantly increases the expression of the c-myc protooncogene (Murphy et al. 1987). Accelerated growth of osteogenic sarcoma was found in patients with elevated levels of GH and somatomedins (Ward et al. 1987). In addition, their have been reports of increased GH concentrations associated with bone cancer (Ratner and Hare 1983) and multiple myeloma (Haegg and Asplun 1988). Human neoplastic lung tissue, freshly obtained at surgery, contained more IGF-I than did the corresponding normal tissue (Minuto et al. 1986). Genetically increased serum levels of GH in transgenic mice result in splanchnomegaly coupled with glomerular sclerosis and hepatocellulamegaly. In contrast, with the exception of selective enlargement of organs, the chronic

expression of IGF-I results in a different pattern of abnormalities, suggesting that the pathogenesis of GH-related disorders is not mediated solely by IGF-I (Quaife et al. 1989), although GH regulation of IGF-I gene transcription is rapid (Doglio et al. 1987). Lowe et al. (1987) showed that the expression of the alternative 5′-untranslated region in the rat IGF-I gene is differentially regulated by GH in various tissues. The GH dependency of IGF-I levels has been amply confirmed in humans. In patients with complete GH deficiency, IGF-I levels are always decreased, the lowest levels being found in patients with Laron dwarfism who lack GH receptors (Daughaday and Trivedi 1987). The administration of GH induces a rise in IGF-I in GH-responsive patients. However, it is not necessary to invoke colocalisation of IGF-I and GH receptor expression because the role of GH in tumour cells may be to regulate the expression of mature cell function rather than to promote cellular proliferation through local IGF-I synthesis. These two hormones do not always act in series; in some tissues, IGF-I is synthesised independently of GH (Hynes et al. 1987), despite the fact that these tissues possess GH receptors, as evidenced by potent mitogenesis independent of IGF-I in response to GH (Rabonovitch et al. 1983). Whether GH can act independently of IGF-I synthesis in human tumour cells remains to be established.

GH in serum has been shown to circulate complexed with GHBP (Baumann et al. 1986; Herington et al. 1986). GHBP exists in rabbit, human and rat serum and contains the extracellular portion of the GH receptor. In humans and rabbit, the GHBP can be produced by specific proteolytic cleavage of the receptor extracellular domain (Edens et al. 1994). It is theorised that in the mouse and rat, GHBP is derived from the GH receptor gene by an alternative mRNA splicing mechanism such that the transmembrane and intracellular domains of the GH receptor are replaced by a hydrophilic carboxyl group (Leung et al. 1987). The extracellular hormone binding protein is therefore common to both the GH receptor and the GHBP. The MAbs used in this study recognise epitopes shared by both the GH receptor and the GHBP, thus, no distinction between the location of the GH receptor and the GHBP was possible. However, the distribution of GHBP immunoreactivity is identical to that for the extracellular region of the GH receptor. Because of the similar affinity of the circulating serum GHBP and the GH membrane receptor, it seems probable that the former has a role in the regulation of GH delivery to tissues. The identity of the extracellular domain of the GH receptor and the serum GHBP has been confirmed by sequencing (Leung et al. 1987). In rats, the GHBP has been shown to restrict human GH to the vascular space and lowers its rate of clearance sixfold (Baumann et al. 1987).

Although immunoreaction was predominantly cytoplasmic, strong nuclear expression was also apparent in many instances. Staining was heterogeneous and varied with the stage of tissue development, sometimes being associated with heterochromatin. MAbs 43 and 263 were immunoreactive in identical locations and in some rat/rabbit tissues immunoreactivity with MAbs 1 and 5 could also be detected. Not all cells displaying cytoplasmic localisation of the chromogen possessed nuclear immunoreactivity. The nuclear localisation of immunoreactivity obtained is the result of nuclear GH receptor/binding protein, identically to the cytosolic and plasma GHBP, using our panel of five MAbs to the GH receptor extracellular region. The nuclear receptor corresponds to the GHBP produced by cleavage or alternative mRNA splicing (Waters et al. 1990). Its role in both the cytoplasm and nucleus is not known, but may be part of a mitogenic mechanism. The presence of receptors in the nucleus suggests that GH internalised to the nucleus may bind to chromatin GHBP and modulates transcription of GH-specific mRNA. GH must be transported to the nucleus if it is to exert its action via nuclear receptors. The localisation in the nucleus of the receptor intracellular domain seems likely to be a requirement for GH to have a nuclear function. Lobie et al. (1994) characterised the rat nuclear GH receptor and binding protein from isolated liver nuclei and showed that the full-length receptor is associated with the nucleus, including the subnuclear fractions of nucleoplasm, outer and inner nuclear membranes and chromatin. The nuclear chromatin shares two identical determinants with the membrane receptor and the nuclear GH receptor/binding protein was identical in physico-chemical and antigenic characteristics to the cytosolic GHBP found in rabbit liver nuclei and associates with the nuclear chromatin (Lobie et al. 1991). Localisation of GH receptor immunoreactivity in the membrane/cytoplasm and the nucleus of cells suggests that receptor-mediated intracellular GH transport is possible. The nuclear chromatin-associated GH receptor may be involved in transcriptional regulation. Recent work by Baumbach et al. (1989) revealed the existence of two related GH receptor mRNAs in mouse liver and adipose tissue, and they proposed alternative mRNA splicing as a mechanism for generating heterogenous forms of the GH receptor and the serum GHBP from a single gene. Although no function of the nuclear GH receptor can be ascribed on the basis of our observations, the heterogeneity of the GH receptor nuclear immunoreaction suggests a relationship of the nuclear receptor to specific cell events such as the cell cycle (D.T. Lincoln et al., in preparation). A functional significance of the nuclear GH receptor and nuclear translocation of GH has not yet been demonstrated and further investigation may establish a direct nuclear reaction of GH in signal transduction. If the nuclear receptor is to be of functional significance, the presence of the intracellular domain of the receptor in the nucleus is presumably necessary. The GH receptor has not yet been implicated in oncogenesis, although it shares two properties of protooncogeneses: the length of the 5′-untranslated region of the rat receptor cDNA is significantly longer than average and it possesses two transcription start sites upstream of the presumed initiator of translation. Both features are common in mRNA coding for protooncogenes. Essential for the initiation of

cellular response to GH, the presence of receptors for this hormone does not necessarily predict the type of biological response resulting from GH exposure. Correlation between the presence of GH receptors and proliferation of human tumours has yet to be proven. Nevertheless, the use of antibodies to the GH receptor to block tumour progression is an intriguing possibility, as is the use of long-acting somatostatin analogues.

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