Insulin-like Growth Factor I Expression by Tumors of Neuroectodermal Origin with the t(11;22) Chromosomal Translocation

A Potential Autocrine Growth Factor

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Abstract

Expression of insulin-like growth factor I (IGF-I) mRNA by some tumor cell lines of neuroectodermal origin has been described. To further explore the significance of IGF-I mRNA expression in these tumors, a more extensive analysis was performed. Most (9 of 10) neuroectodermal tumor cell lines with a t(11;22) translocation (primitive neuroectodermal tumor [PNET], Ewing's sarcoma, esthesioneuroblastoma) expressed IGF-I mRNA, whereas 0 of 15 cell lines without the translocation (PNET, neuroblastoma) expressed IGF-I. Furthermore, inasmuch as all neuroblastoma (12 of 12) cell lines examined expressed IGF-II RNA, the pattern of IGF expression could distinguish between these closely related tumors. CHP-100, a PNET cell line with the t(11;22) translocation, was shown to secrete both IGF-I protein and an IGF binding protein, IGFBP-2. This cell line also expressed the type I IGF receptor mRNA, and blockade of this receptor by a monoclonal antibody (αIR3) inhibited serum-free growth. These data demonstrate that IGF-I expression is a property of neuroectodermal tumors with a t(11;22) translocation and that interruption of an IGF-I autocrine loop inhibits the growth of these tumor cells. (*J. Clin. Invest.* 1990. 86:1806–1814.) Key words: Ewing's sarcoma • insulin-like growth factor binding protein 2 • insulin-like growth factor II • neuroblastoma • type I insulin-like growth factor receptor

Introduction

The autocrine growth hypothesis states that malignant cells can produce and secrete polypeptide growth factors that will bind to their own cell surface receptors and stimulate proliferation (1). In order to prove that a secreted growth factor acts via an autocrine pathway, there must be demonstration that the ligand and its receptor are expressed by the cell, that interference with the ligand–receptor interaction inhibits tumor cell growth, and that the growth inhibition caused by this interference is specific. Although growth factors have been reported to function as autocrine growth stimulators in several types of solid tumors (2–5), direct demonstration of these points, especially the specificity of the method used to interrupt the autocrine loop, has been lacking.

Insulin-like growth factor I (IGF-I)[1] is a growth factor important in normal growth and development and is expressed by many normal cells of mesenchymal origin (6). IGF-I has been reported to be an autocrine growth factor for a variety of tumors (7–11); however, using a ribonuclease (RNase) protection assay, we have found that authentic IGF-I mRNA is rarely expressed in tumor cell lines of epithelial origin (12). Previous reports of IGF-I expression may be spurious, owing to the nonspecific detection of cross-reacting mRNAs by the IGF-I cDNA and/or to interference with the IGF-I radioimmunoassay (RIA) by IGF binding proteins.

Neuroblastoma and primitive neuroectodermal tumors (PNETs, also known as peripheral neuroepithelioma) are extracranial tumors that originate from primitive neuroectoderm (13). Although these small round cell tumors can be histologically confused, they may be distinguished from one another by biochemical markers, cell surface antigens, ultrastructural features, patterns of oncogene expression, and presence or absence of a t(11;22)q(12:24) chromosomal translocation (13–20). For example, neuroblastomas express enzymes for catecholamine synthesis and often have amplification of the N-*myc* oncogene. In contrast, PNETs do not express adrenergic enzymes, have low levels of N-*myc* expression, do not have N-*myc* genomic amplification, express choline acetyl transferase, and often have a characteristic t(11;22) chromosomal translocation. This translocation can be seen in other tumors, including Ewing's sarcoma, suggesting that this tumor also arises from a neuroectodermal progenitor (13–15). We have previously reported that some neuroectodermal tumor cell lines bearing this chromosomal translocation expressed IGF-I mRNA (12). In this study, we have examined a large number of cell lines derived from extracranial neuroectodermal tumors for IGF-I and IGF-II mRNA expression. We also have explored whether IGF-I could regulate the growth of these tumors via an autocrine pathway.

Methods

Materials. Recombinant IGF-I was purchased from Amgen, Thousand Oaks, CA. ^{125}I-IGF-I was purchased from Amersham Corp., Arlington Heights, IL. The monoclonal antibody αIR3 was a gift from S. Jacobs, Burroughs Wellcome Co., Research Triangle Park, NC. Antiserum to the MDBK binding protein was a gift from F. Ballard and J. Wallace, University of Adelaide, South Australia. The antibody UBK 487 was obtained from the National Pituitary Agency. The IGF-I, IGF-II, and the type I IGF receptor cDNAs were kindly provided by K. Gabbay, Baylor College of Medicine, Houston, TX; G. Bell, Howard

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1. *Abbreviations used in this paper:* IGF-I, insulin-like grow factor I; PNET, primitive neuroectodermal tumor; RNase, ribonuclease.

Hughes Institute, Chicago, IL; and A. Ullrich, Max Planck Institute, Munich, Germany; respectively. Cell lines were kindly provided by Children's Hospital of Pennsylvania (CHP-100), T. Triche, Children's Hospital of Los Angeles, Los Angeles, CA (TC-32, TC-106, TC-135, A4573, A9423, 5838, 6647), J. Biedler, Memorial Sloan-Kettering Cancer Center, (SK-N-SH, SK-N-MC, SK-N-BE[1]), and L. Helson, New York Medical College, Valhalla, NY (SK-PN-LI, SK-PN-DW, SK-N-RA, SK-M-AS, SK-N-FI).

Cell lines. The cell lines were maintained in RPMI 1640 and 10% heat-inactivated fetal calf serum in a humidified atmosphere containing 5% CO_2. Cell lines established from neuroectodermal tumors containing the t(11;22) translocation are PNETs (CHP-100, SK-N-MC, TC-135, and TC-32), esthesioneuroblastoma (JFEN), and Ewing's sarcomas (TC-106, 5838, A4573, A9423, 6647) (13–16, 21–23). CB-AGPN, SK-PN-DW, and SK-PN-LI are PNET cell lines without the t(11;22) chromosomal translocation (18, 24) (J. Whang Peng, personal communication). SK-N-RA, SK-N-SH, SMS-LHN, LA-N-6, SK-N-AS, and SK-N-FI are neuroblastoma cell lines that lack N-*myc* genomic amplification (18, 23, 25). SMS-KAN, SMS-KCN, SMS-KCNR, SMS-SAN, SMS-MSN, and SK-N-BE(1) are N-*myc* amplified neuroblastoma cell lines (18, 26, 27).

RNase protection assays. RNase protection assays were performed as previously described (28). Briefly, 30 μg of total RNA were hybridized with labeled anticomplementary RNA probes transcribed from pGem vectors (Promega Biotec, Madison, WI) in solution at 50°C for 12–18 h. The RNA was digested with 40 μg/ml of RNase A (Sigma Chemical Co., St. Louis, MO) for 30 min at 25°C. The protected fragments were fractionated on a 6% polyacrylamide gel and exposed to X-ray film in the presence of an intensifying screen for 12–18 h at −70°C.

The IGF-I probe was transcribed from a 519-bp EcoRI-BamHI IGF-I cDNA. This probe contains most of the coding region of the IGF-I cDNA (12). A 336-bp RsaI-PstI fragment of the IGF-II cDNA was used to transcribe the probe which contains portions of the E domain and 3′ untranslated region of IGF-II (28). To transcribe the type I IGF receptor probe a 293-bp SmaI-AvaI cDNA that encodes the transmembrane domain of the β chain was used as a template (29).

Gel filtration and IGF-I RIA. CHP-100 cells were plated in improved minimal essential medium (IMEM, Gibco Laboratories, Grand Island, NY) with 10% fetal calf serum (Biofluids, Rockville, MD) for 24 h, rinsed three times with phosphate-buffered saline, and refed with serum-free medium (IMEM plus 292 mg/liter glutamine, trace elements, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, 2 mg/liter transferrin, and 2 mg/liter fibronectin). After 48 h the conditioned medium was collected in the presence of protease inhibitors (aprotinin, phenylmethylsulfonyl fluoride, pepstatin A, and leupeptin) and concentrated 100-fold with an ultrafiltration apparatus (Amicon Corp., Danvers, MA) using a 5,000 mol wt cutoff filter (YM5). 1 ml of the concentrated conditioned medium was added to an equal volume of 2 M acetic acid and incubated at 4°C overnight. The medium was then fractionated on a 1.5 × 90-cm Sephadex G100 column by eluting with 1 M acetic acid. 2-ml fractions were collected, lyophilized, and reconstituted in water. IGF-I RIA using the antibody (UBK 487) and IGF charcoal separation binding protein assays were performed on each fraction as previously described (30, 31). Bovine serum albumin, ovalbumin, chymotrypsin, and RNase were used as molecular weight markers (Bio-Rad Laboratories, Richmond, CA).

IGF binding protein ligand and immunoblots. 1 ml of 100-fold concentrated conditioned medium was further concentrated to 40 μl by ultrafiltration, separated by 12% SDS-PAGE electrophoresis, and transferred from the gel to nitrocellulose. IGF binding proteins were identified by incubating the membrane with ^{125}I-IGF-I (Western ligand blot) as previously described (32). Bovine serum binding proteins were isolated by a previously described method (33) and ~ 250 ng of each binding protein was applied to the same gel as the CHP-100 conditioned media.

Western immunoblotting was performed using an antiserum directed against MDBK-BP, the IGF binding protein produced by MDBK (normal bovine kidney) cells (34). 500 ng of MDBK-BP, human IGFBP-1 (hBP-25), each serum bovine binding protein, and 40 μl of 2,500-fold concentrated CHP-100–conditioned medium were fractionated on a 12% SDS-PAGE gel under nonreducing conditions. The proteins were transferred to nitrocellulose and incubated with a 1:500 dilution of the MDBK antiserum and developed as previously described (35).

Cell growth experiments. CHP-100 were plated in triplicate in 12-, 24-, or 48-well dishes in IMEM with 10% fetal calf serum at cell densities of 20–40,000 cells per well. SK-PN-DW were plated in 24-well dishes at 20,000 cells per well. On day 1 the cells were washed three times with phosphate-buffered saline and then 1 ml of serum-free medium (as noted above) with 26 μg/ml isotypic mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), αIR3 (26 μg/ml), or 5 nM IGF-I was added. On day 3, IgG, αIR3, and IGF-I were again added to the medium, however the medium was not changed. Cells were counted by hemocytometer on days 1, 3, and 5.

Results

IGF-I, IGF-II, and type I IGF receptor mRNA expression. RNase protection assays using an IGF-IA cDNA probe demonstrated that only PNET cell lines with a t(11;22) translocation (CHP-100, SK-N-MC, TC-135, TC-32), an esthesioneuroblastoma with a t(11;22) translocation (JFEN), and Ewing's sarcomas with a t(11:22) translocation (TC-106, 5838, A9423, A4573) expressed IGF-I mRNA (Fig. 1, *A* and *B* and Table I). In contrast, neither PNET cell lines lacking the translocation (SK-PN-LI, SK-PN-DW, CB-AGPN) or neuroblastoma (SK-N-RA, SK-N-SH, SMS-LHN, LA-N-6, SK-N-AS, SK-N-FI) expressed IGF-I mRNA. Additionally, one Ewing's sarcoma cell line reported to have the t(11;22) translocation (6647) failed to express IGF-I mRNA. Thus, 9 of 10 neuroectodermal tumor cell lines with the t(11;22) translocation expressed IGF-I mRNA whereas none of the cell lines (0/15) lacking the translocation showed IGF-I mRNA expression.

Similar studies using an IGF-II cDNA in RNase protection assay showed that all neuroblastoma cell lines (12 of 12) examined expressed IGF-II mRNA (Fig. 1 *C* and Table I). Both N-*myc* amplified and nonamplified cell lines expressed IGF-II mRNA. None of the IGF-I expressing neuroectodermal tumor cell lines known to have the t(11;22) chromosomal translocation expressed IGF-II mRNA. Therefore, the pattern of IGF expression distinguished between closely related neuroectodermal tumors.

All cell lines examined expressed type I IGF receptor mRNA (Fig. 2, *A* and *B*, Table I). Since the type I IGF receptor is thought to mediate the mitogenic effects of IGF-I (36), the expression of both ligand and receptor by neuroectodermal tumors with t(11;22) translocation suggest that an autocrine loop could exist. In the cell line CHP-100, the addition of exogenous 5–10 nM IGF-I stimulated the growth of the neuroepithelioma cells, under serum-free conditions (see Figs. 5 and 6). This demonstrates that the type I IGF receptor mRNA detected in this cell line is translated into a receptor capable of mediating a mitogenic response.

IGF-I and IGF binding protein expression by CHP-100. We next evaluated the PNET cell line CHP-100 for expression of IGF-I peptide. Concentrated serum-free conditioned media was examined for IGF-I activity by RIA after gel filtration chromatography. Under neutral conditions, the peak of radioimmunoassayble material eluted with a molecular mass of 50 kD (data not shown). This is compatible with binding of IGF-I to a higher molecular mass binding protein and/or interference of IGF binding protein with the RIA. We have previously

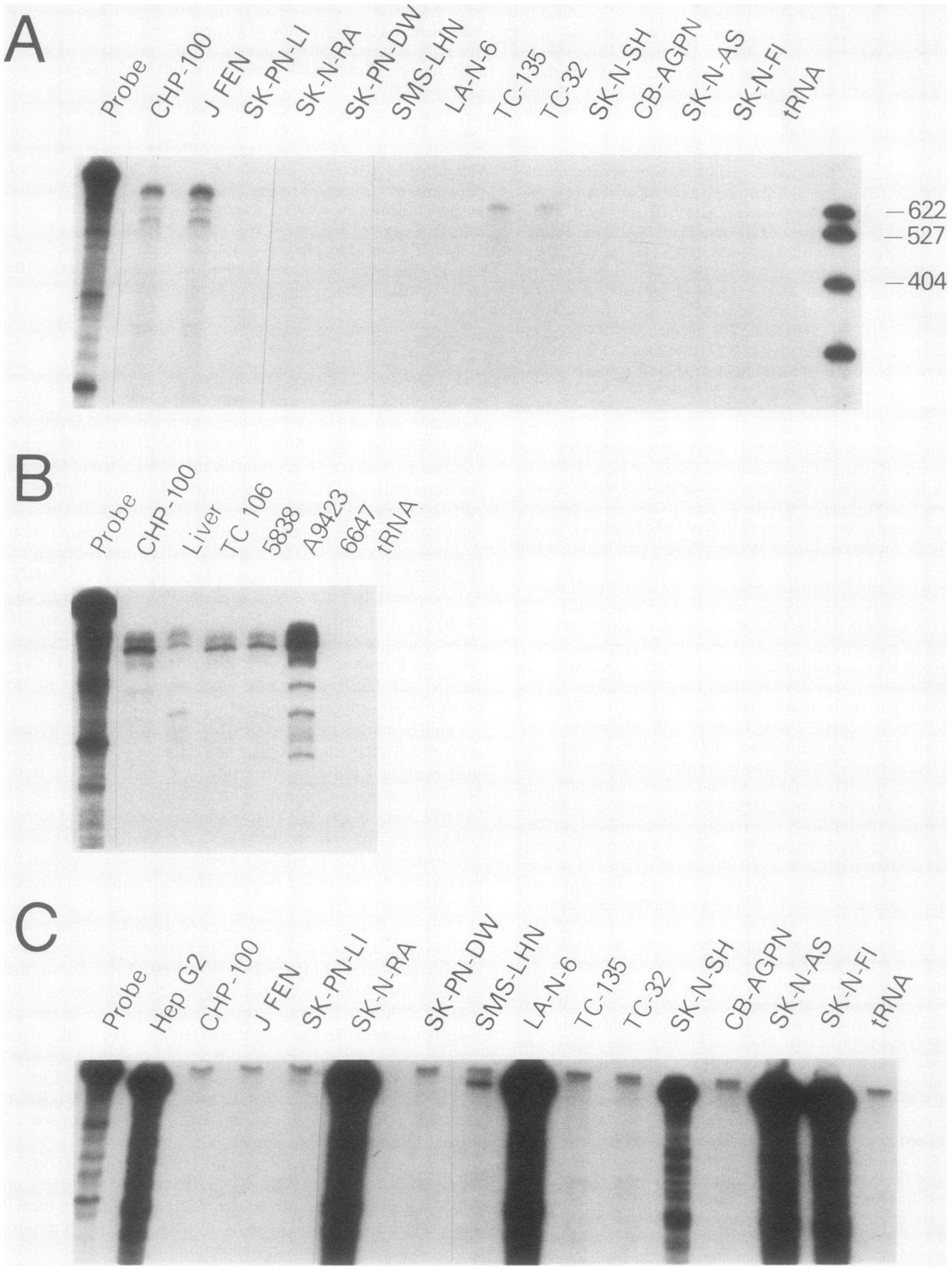

Figure 1. IGF-I and IGF-II RNase protection assays in tumor cell lines. 30 μg of total RNA was used in each lane. (*A*) IGF-I mRNA expression in PNET cell lines. Only neuroepithelioma or esthesioneuroblastoma cell lines with the t(11;22) translocation expressed IGF-I mRNA (CHP-100, TC-35, TC-132, JFEN). (*B*) IGF-I mRNA expression in Ewing's sarcoma cell lines. All Ewing's sarcoma cell lines (TC-106, 5838,

Table I. Cell Lines and Relative IGF mRNA Expression Determined by RNase Protection Assay

Cell line	IGF-I mRNA	IGF-II mRNA	Type I IGF receptor mRNA
PNET with t(11;22) translocation			
CHP-100	++	−	++
SK-N-MC	+++	−	++
TC-32	+	−	++
TC-135	+	−	++
Esthesioneuroblastoma with t(11;22)			
JFEN	++	−	+++
Ewings' sarcoma with t(11;22)			
A4573	+	−	+
TC-106	+	−	+++
5838	+	−	+++
A9423	+++	−	+
6647	−	−	++
PNET without t(11;22)			
SK-PN-LI	−	−	++
SK-PN-DW	−	−	+
CB-AGPN	−	−	+
Neuroblastoma without M-myc amplification			
SK-N-RA	−	+++	++
SMS-LHN	−	+	+++
LA-N-6	−	+++	++
SK-N-SH	−	++	++
SK-N-AS	−	+++	+
SK-N-FI	−	+++	++
Neuroblastoma with N-myc amplification			
SMS-SAN	−	+	+++
SMS-KCN	−	+	+++
SMS-MSN	−	+++	++
SMS-KAN	−	+	+++
SK-N-BE(1)	−	+	++
SMS-KCNR	−	+	++

For IGF-I mRNA and type I IGF receptor mRNA, ++ equals the level of expression found in CHP-100. For IGF-II, ++ equals SK-N-SH expression. mRNA expression was judged as either more (+++), equal (++), less (+), or not detected (−) when compared to these standards.

noted that breast cancer cell lines produce a binding protein of this approximate molecular mass and that this binding protein interferes with the IGF-I RIA (30). To separate IGF binding activity from IGF-I, we treated the conditioned media with 1 M acetic acid and chromatographed under acid conditions. The eluted fractions were neutralized and IGF-I RIA performed on each fraction demonstrated two peaks of activity (Fig. 3). The larger peak centered close to 50 kD was compatible with the molecular mass of some IGF binding proteins.

The smaller peak at ~ 14–10 kD was compatible with the size and elution position for authentic IGF-I. Using an identical gel filtration column, we previously demonstrated that the elution position of authentic radiolabeled IGF-I was found in a similar range of column fractions (30). In addition, we examined the fractions for IGF-I binding protein using the charcoal separation assay and found that after acid treatment, only the larger molecular mass fractions contained IGF binding activity (Fig. 3). The smaller peak had no binding protein activity detectable by charcoal separation assay. Therefore, the smaller peak represents authentic immunoreactive IGF-I protein produced by CHP-100.

In order to identify the species of IGF binding protein contained in the larger peak, we performed Western ligand blots on concentrated CHP-100 conditioned media (Fig. 4). The migration positions of the detected binding protein species were compared with those of purified bovine binding proteins detected by India ink staining (lanes *8* and *9*). Fig. 4 demonstrates that CHP-100 produced an IGF binding protein species of 34 kD (lane *3*) that comigrated with a 34-kD binding protein detected in bovine serum (lanes 2 and 8). Additionally, minor bands at 28 and 24 kD could also be detected in CHP-100–conditioned media (Fig. 4. and data not shown). The M_r of the major binding protein species suggested that it may be IGFBP-2 (37, 38) which has been described as present in cerebrospinal fluid (37, 39).

To further demonstrate that CHP-100 expressed IGFBP-2 we used an antisera directed against the MDBK (normal bovine kidney cells) IGF binding protein (bovine IGFBP-2) (34) in Western immunoblot. Fig. 4 demonstrates that this antisera detected 34-kD purified bovine IGFBP-2 (lane *4*), and a 34-kD band in bovine serum (lane *7*) and CHP-100–conditioned media (lane *6*). Therefore, the IGF binding protein is immunologically related to bovine IGFBP-2. Since the MDBK and human IGF (IGFBP-2) binding proteins are homologous (34, 38), these observations suggest that the 34-kD binding protein produced by CHP-100 is human IGFBP-2. Therefore, the larger IGF-I immunoreactive peak detected on RIA of the gel-filtered CHP-100–conditioned media was most likely produced by artifactual interference of IGFBP-2 with the IGF-I RIA.

Growth effects of type I IGF receptor blockade. Since CHP-100 expressed IGF-I, IGFBP-2, and type I IGF receptor mRNA, we examined the possibility that IGF-I could serve as an autocrine growth factor for this tumor by studying the effects of antibody blockade of the receptor. We grew the cells in the presence of 26 μg/ml of an antibody (αIR3) directed against the type I IGF receptor (40). αIR3 has been shown to block the binding of IGF-I to the type I IGF receptor at a concentration of 13 μg/ml without eliciting a biological response (36, 41). Under serum-free conditions, this concentration of antibody inhibited the growth stimulatory effects of exogenous IGF-I on CHP-100, but was ineffective at decreasing basal growth (data not shown). However, higher concentrations of αIR3 decreased cell number by 25–50% after 5 d of treatment when compared to control (Figs. 5 and 6). Nonspe-

A9423) except 6647 expressed IGF-I mRNA. (*C*) IGF-II mRNA expression in PNET cell lines. In each lane, a band larger than the fragment protected by IGF-II mRNA is seen. The larger band represents probe resistant to digestion and has been previously described (28). Only neuroblastoma cell lines (SK-N-RA, SK-N-SH, SMS-LHN, LA-N-6, SK-N-AS, SK-N-FI) expressed IGF-II mRNA. HepG2 is a hepatoblastoma cell line.

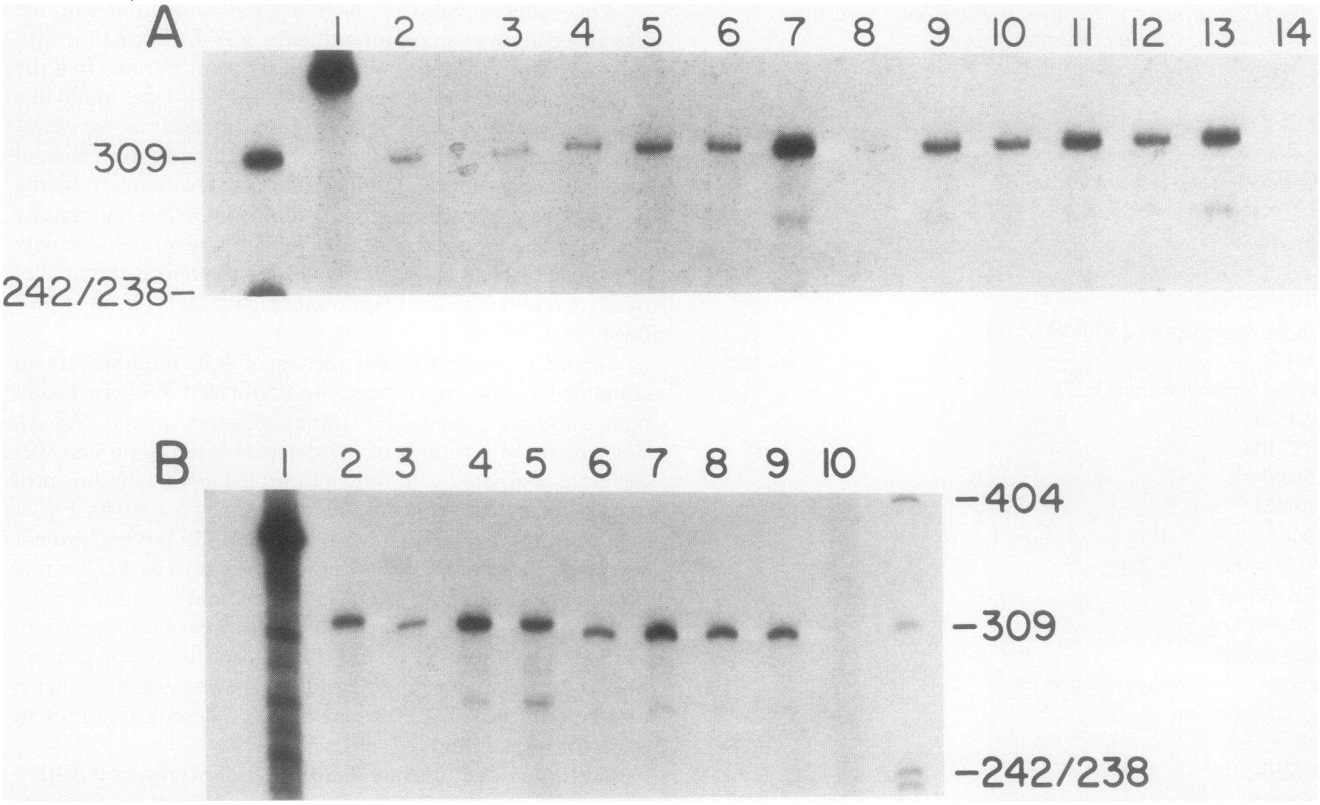

Figure 2. Type I IGF receptor RNase protection assay. A 293-bp probe was used as described in Methods. (*A*) Type I IGF receptor mRNA expression in PNET, Ewing's sarcoma, and neuroblastoma cell lines. Lanes are: *1,* undigested probe; *2,* CHP-100, *3,* TC-135; *4,* TC-32; *5,* TC-106; *6,* 5838; *7,* A9423; *8,* 6647; *9,* SK-PN-LI; *10,* SK-N-RA; *11,* SMS-LHN; *12,* SK-N-SH; *13,* SK-N-FI; *14,* tRNA. (*B*) Type I IGF receptor in PNET and N-*myc* amplified neuroblastoma cell lines. Lanes are: *1,* undigested probe; *2,* CB-AGPN; *3,* SK-PN-DW; *4,* SMS-SAN; *5,* SMS-KCN; *6,* SMS-MSN; *7,* SMS-KAN; *8,* SK-N-BE(1); *9,* SMS-KCNR; *10,* tRNA.

cific isotypic IgG (26 μg/ml) had no effect on the growth of the cells (Figs. 5 *A* and 6). Exposure of the cells to exogenously added IGF-I (10–100 nM) after αIR3 treatment reversed the growth inhibition of αIR3 (Fig. 6) and cells grew at control levels. Although the highest concentration of IGF-I resulted in growth stimulation, this result did not achieve statistical significance (Fig. 6, expt. 4). Additionally, αIR3 had no effect on the growth of SK-PN-DW, a PNET cell line which expressed type I IGF receptor mRNA but neither IGF-I or IGF-II mRNA (Figs. 1 *A,* 2, and 5 *B*).

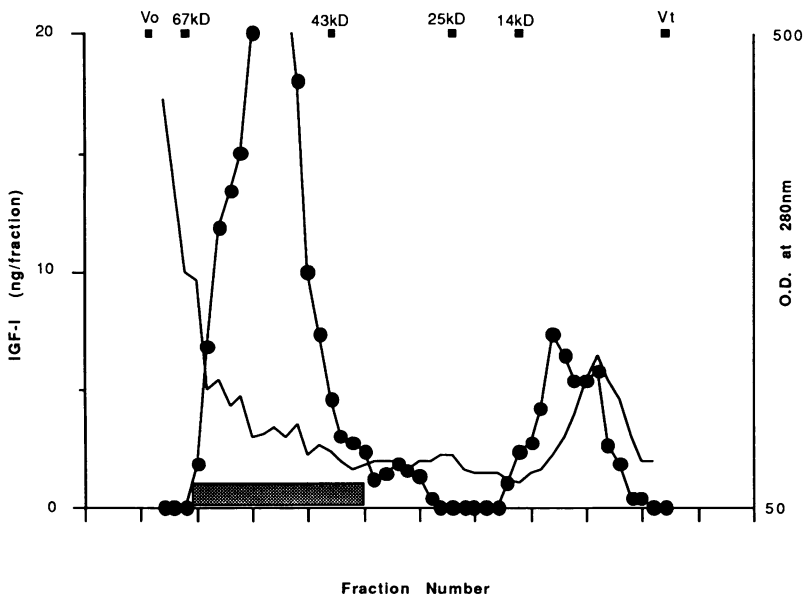

Figure 3. Acid gel filtration chromatography of CHP-100–conditioned media. Each fraction was assayed for IGF-I by RIA and for IGF binding protein activity by the charcoal separation assay. The solid line represents the total protein as measured by the optical density at 280 nM; The solid circles represent IGF-I immunoreactive material detected by RIA; and the shaded box shows the range of IGF binding protein activity. The *x*-axis is marked by 10 fraction increments.

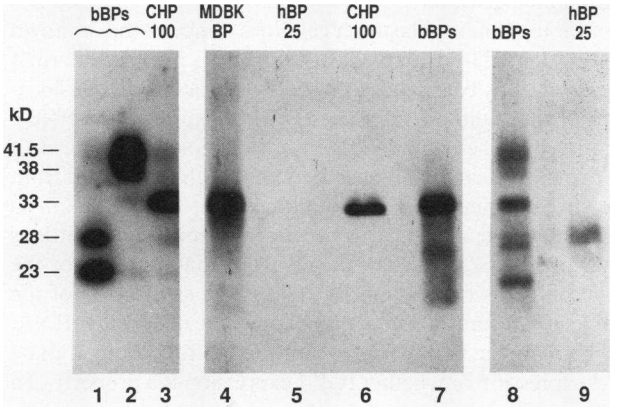

Figure 4. Western ligand and immunoblot of CHP-100–conditioned media and purified bovine IGF binding proteins. Western ligand blot (lanes *1–3*) using labeled IGF-I demonstrates the migration positions of the purified bovine IGF binding proteins at 23 and 28 kD (lane *1*) and 34, 38, and 41.5 kD (lane *2*). CHP-100 (lane *3*) expressed a major 34-kD band, although minor bands at 23 and 28 kD were also seen. Western immunoblot (lanes *4–7*) using the antiserum directed against the MDBK binding protein (bovine IGFBP-2) demonstrated specific detection of IGFBP-2 in purified MDBK BP (lane *4*), CHP-100–conditioned media (lane *6*), and purified bovine binding proteins (lane *7*). Human IGFBP-1 (BP-25, lane *5*) and other larger molecular mass purified bovine binding proteins (lane *7*) were not detected by this antiserum. However, some cross-reactivity between the 28- and 23-kD binding protein species was seen (lane *7*). India ink staining of purified bovine binding proteins and IGFBP-1 is shown in lanes *8* and *9* for comparison to the migration pattern seen in Western ligand and immunoblot.

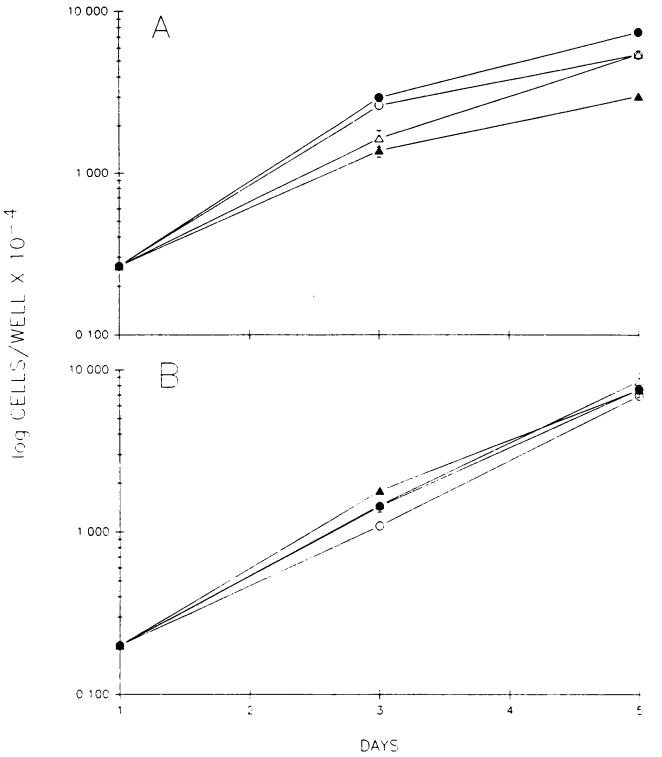

Figure 5. Growth effects of IGF-I and αIR3 on CHP-100 and SK-PN-DW plotted on a log scale. (○) Serum-free media (SFM) alone; (△) SFM plus nonspecific mouse IgG; (●) SFM plus 5 nM IGF-I; (▲) SFM plus 26 μg/ml αIR3. Error bars represent standard error of the mean. (*A*) CHP-100, a PNET cell line that expresses IGF-I; (*B*) SK-PN-DW, a PNET cell line that does not express IGF-I.

The results of five individual experiments are demonstrated in Fig. 6. αIR3 caused statistically significant growth inhibition of CHP-100 cells when compared to their basal growth in serum-free media. Since excess ligand could reverse this effect and no growth inhibition was demonstrated in a non-IGF producing cell, this suggests that interruption of the IGF-I ligand–receptor interaction by αIR3 blockade of the receptor caused specific growth inhibition of CHP-100.

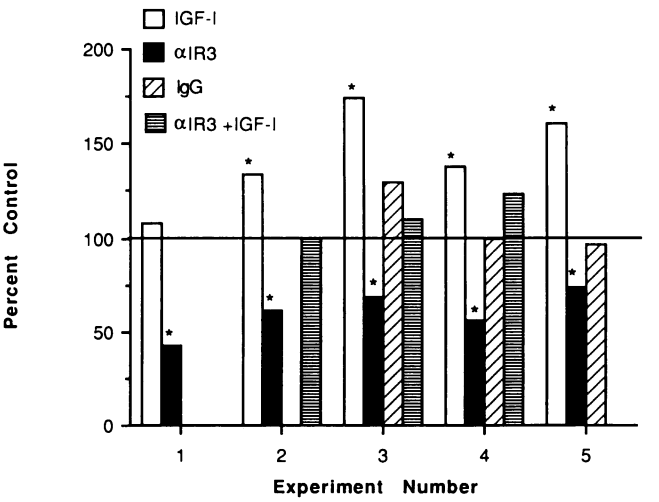

Figure 6. Growth inhibition of CHP-100 cells by αIR3. Bars represent the percent control growth of each treatment group when compared to growth in serum-free media after 5 d of exposure. Open bar shows growth in 5 nM IGF-I; solid bar shows growth in 26 μg/ml αIR3, the vertically cross-hatched box shows growth in 26 μg/ml nonspecific IgG; and the horizontally cross-hatched box shows growth in the presence of αIR3 and additional IGF-I. The concentrations of the IGF-I added to αIR3 (horizontally cross-hatched box) are 5 nM in expt. 2, 20 nM in expt. 3, and 100 nM in expt. 4. Student's *t*-test was performed on each treatment group and compared to control from each experiment. Asterisk identifies treatments that were significantly ($P < 0.08$) different from control.

Discussion

In the human central nervous system the expression of both IGF-I and -II is low (23, 42, 43) although little is known about the pattern of IGF expression in the peripheral nervous system. However, in proliferating embryonic neurons isolated from rodent brain, IGF-I mRNA expression has been reported (44). Based on this observation, it has been suggested that IGF-I may be an autocrine growth factor for proliferating neuroblasts. IGF-I has been shown to be an autocrine growth factor for normal rodent glial cells (45). In other epithelial cells, studies in nontransformed breast cancer cells and colon cells line have demonstrated that an analogous autocrine pathway, composed of a TGFα/EGF receptor loop, is operative (46, 47). These data support the concept that autocrine pathways are not only a feature of malignant epithelial cells, but may also be found in normal or premalignant proliferating cells. A lack of regulation or an alteration in the response to these pathways may be important in the unregulated cellular proliferation that characterizes cancer. It is possible that unregulated IGF-I expression by tumors derived from neural pre-

cursors may play a role in the autocrine growth control of the tumor.

We have demonstrated that a subset of tumors derived from the neuroectoderm express IGF-I mRNA. Furthermore, a representative cell line, CHP-100, expressed IGF-I and an IGF binding protein. It has been suggested that some IGF binding proteins may act to augment or inhibit the mitogenic effects of IGF-I (48–50). It seems likely that expression of IGF binding proteins along with expression of IGF-I could be important in the autocrine growth regulation of the tumor. Of note is that IGFBP-2 contains an Arg-Gly-Asp (RGD) sequence which may be important in cell surface binding (38) and could influence the interaction between IGF-I and its receptor. Further study of the functions of the IGF binding proteins should clarify their roles in modulating IGF action.

We have shown that blockade of the type I IGF receptor with the monoclonal antibody αIR3 inhibited CHP-100 proliferation. However, because αIR3 alone causes serine phosphorylation of the type I IGF receptor (51), this antibody may induce some incompletely characterized physiological effects. αIR3 has been reported to have antiproliferative effects in small-cell lung cancer (7), pancreatic cancer (11), and neuroblastoma (5), but it is unclear if these results are specifically due to the interruption of an IGF-mediated autocrine loop. We have found that αIR3 did not inhibit the growth of a PNET (SK-PN-DW) cell that expressed the type I IGF receptor but did not express IGF-I or IGF-II, that nonspecific IgG did not inhibit CHP-100 growth, and that the effects of αIR3 on CHP-100 could be partially reversed by excess IGF-I. These observations support the concept that the antiproliferative effects of αIR3 on CHP-100 cells were specific and due to its ability to block the IGF-I ligand–receptor interaction, and suggest that IGF-I is an autocrine growth factor for these PNET cells.

Interruption of the IGF-I autocrine loop required higher concentrations of αIR3 than those needed to block the effects of exogenously added IGF-I. In addition, the inhibition caused by antibody is only partial and excess IGF-I added in the presence of αIR3 could not stimulate growth above control levels. Several factors may account for these observations. The production of IGF binding proteins may alter the receptor's affinity for IGF-I. If IGF binding proteins enhance the ability of the type I IGF receptor to bind IGF-I, then it may be more difficult to interrupt this ligand–receptor interaction by αIR3. It is possible that IGF-I may bind and activate its receptor intracellularly as has been described for platelet-derived growth factor (52). This would lead to activation of the type I IGF receptor that could not be interrupted by an antibody delivered to the extracellular space. In addition, IGF-I may be bound to its receptor as the receptor is transported to the cell surface. In either of these conditions, it could be postulated that higher concentrations of antibody would be needed to interrupt this "pre-bound" ligand–receptor interaction than would be needed to block exogenous ligand. These possibilities are being examined.

Using Northern blot analysis, El-Badry et al. (5) demonstrated that neuroblastoma cells expressed IGF-II mRNA. We have used RNase protection assay, a more sensitive and specific method of detecting IGF mRNA transcripts (12, 53), and have confirmed that 12 of the neuroblastoma cell lines we studied express IGF-II. Since the panel of neuroblastoma cell lines we studied included both N-*myc* gene amplified and nonamplified cells, these data suggest that IGF-II expression is common to all neuroblastoma cell lines. Since we have shown that IGF-I mRNA is expressed by 9 of 10 neuroectodermal tumor cell lines bearing the t(11;22) chromosomal translocation and that neither IGF-I or IGF-II is expressed by PNET cell lines that lack the t(11;22) translocation, the pattern of IGF expression can distinguish between cell lines derived from closely related neuroectodermal tumors.

The IGF-I gene is located on chromosome 12 and little is known about the molecular mechanisms that account for the regulation of its expression. Of note is the association of the chromosomal translocation and expression of IGF-I mRNA. It is not known if the translocation acts directly to initiate IGF-I expression or whether IGF-I expression is a property of a cell lineage that frequently develops the translocation during oncogenesis. If the translocation is involved in the activation of the IGF-I gene, this would suggest a mechanism by which this genetic event could be responsible for the autocrine growth stimulation of PNET, and potentially Ewing's sarcoma, by IGF-I. Similarly, the observation that IGF-II is produced by all neuroblastoma cell lines that we examined, suggest that autocrine stimulation of these tumors by IGF-II may be a common event.

Interference with the interaction of IGF-I or IGF-II with the type I IGF receptor in tumors of neuroectodermal origin has now been demonstrated to slow in vitro tumor growth (5). αIR3 mediated type I IGF receptor blockade has also been shown to slow breast cancer cell growth in an in vivo athymic mouse model (54). Taken together, these studies suggest that interference of the IGF receptor–ligand interaction may prove to be an important therapeutic modality for many types of tumors.

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