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GROWTH FACTORS AND FIBROBLAST GROWTH FACTOR RECEPTORS IN CEREBRAL GIGANTISM

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In two children with cerebral gigantism serum growth hormone, somatomedin C, nerve growth factor, and epidermal growth factor levels were unremarkable. Skin biopsy–derived fibroblasts from one child, when grown in confluent monolayer culture, demonstrated epidermal growth factor and somatomedin C receptor concentrations that did not differ from controls. Qualitative growth kinetics of these fibroblasts were also unremarkable.

Serum and plasma protein dialysate from one child significantly stimulated the growth of AKR-2B mouse fibroblasts in tissue culture when compared to controls. This effect was reproducible.

These results indicate that currently assayable growth factors and fibroblast receptors for epidermal growth factor and somatomedin C are unremarkable in cerebral gigantism. The serum and plasma protein stimulation of AKR-2B mouse fibroblast growth in one child may be a demonstration of a novel growth factor in this condition.

Cerebral gigantism as described by Sotos et al. (1964) is a syndrome of macrocephaly, macrosomia, and mental retardation. It is an unusual affliction, in that mental retardation is associated with overgrowth, as contrasted to the more usual microcephaly and poor growth of retarded individuals. This syndrome offers a rare opportunity for investigating biological factors that may account both for abnormal growth and ab-

normal mentation in these individuals and, by extrapolation, in the retarded population more generally.

Previous investigators, including Saenger et al. (1976), have been unable to demonstrate abnormalities of serum growth hormone and somatomedin concentrations in patients with cerebral gigantism. The purposes of this study were to investigate serum- and plasma-derived growth factors using both conventional and novel methodologies, as well as to study the growth kinetics and surface growth factor receptors in skin biopsy–derived fibroblasts from individuals with the syndrome. We were able to demonstrate unusual serum and plasma protein growth-enhancing properties in one child, raising the possibility of a novel growth factor in this condition.

METHODS

Two children with cerebral gigantism were evaluated. Patient 1 was a 19-month-old male. By the age of 4 months his height, weight, and head circumference were just above the 95th percentile, rankings that he continued to maintain. He was slow in his developmental parameters; he spoke his first words at the age of 1 year, and at the time of examination was unable to sit or walk unsupported. During the examination he was noted to have a dolicocephalic skull with proportionately large hands and feet. Psychometric testing revealed his general development to be at the 11-month level, with gross motor abilities at a 5-month level.

Patient 2 was a 10-month-old male. At the time of birth and subsequently, his height, weight, and head circumference were just above the 95th percentile. His subsequent developmental milestones were slightly delayed and at the time of examination were estimated to be at a 7- to 8-month level. His hands and feet were noted to be proportionately large. In neither patient was there a family history of neurological abnormality.

Routine investigations were performed on both children in the clinical chemistry and hematology laboratories of the Mayo Clinic using standard procedures. These investigations consisted of serum sodium, potassium, calcium, phosphate, total protein, glucose, bilirubin, uric acid, creatinine, alkaline phosphatase, glutamicoxalacetic acid transaminase, total thyroxine, urine screen for inborn errors of metabolism, and cerebrospinal fluid analysis (cell count, glucose, and protein). All results on these tests were normal. Opthalmological examination and skull X-rays in patient 1 and electroencephalogram in patient 2 were normal. In both patients cranial computerized tomography revealed an enlarged brain with mildly enlarged ventricles and subarachnoid spaces. In all of the subsequent investigations, the control and patient blood samples and skin biopsies were obtained with informed consent.

RESULTS

In patient 1 fasting serum growth hormone was 16.5 ng/ml, with suppression 2 hours after an oral glucose challenge (8.8 ng/ml); both blood samples were obtained with difficulty while the patient was crying vigorously. Fasting serum somatomedin C (SM-C) concentration, as measured by radioimmunoassay at the Nichols Institute, Los Angeles, California, was 0.23 U/ml, which was within normal limits for the patient's age. Fasting serum nerve growth factor (NGF) was assayed by a qualitative, in vivo bioassay performed by Dr. David Wells in which chick dorsal root ganglia were incubated in the presence of the patient's serum. The rate and extent ot neurite outgrowth with the patient's serum was indistinguishable from that of serum from normal adults. Fasting serum epidermal growth factor (EGF) concentration was determined by radioreceptor assay and found to be identical to that of normal adults.

In patient 1, fibroblast tissue cultures were established from a skin biopsy obtained, under local xylocaine anesthesia, from the right upper thigh. With the cells grown to a confluent monolayer in McCoy's 5A modified medium, supplemented with 10% fetal bovine serum (FBS), EGF receptor assay did not differ significantly from control fibroblasts.

With the cells grown to a confluent monolayer in Dulbecco's modified Eagle's medium (MEM) with 20% FBS, penicillin (100 U/ml), and streptomycin (100 mg/ml), an assay of cellular binding of ^{125}I-SM-C was performed by the methodology described by Rosenfeld and Dollar (in press). The percent specific binding per 10^6 cells in two separate experiments was 16.58% and 8.21%, which was within the normal range for foreskin fibroblasts. Binding percentages in fibroblasts from adult controls run concurrently were 5.81%, 10.57%, 14.82%, 18.35%), and 15.72%, again demonstrating that SM-C binding in the fibroblasts of patient 1 was normal. Furthermore, the binding characteristics of SM-C to the patient's fibroblasts were normal, indicating that there was a normal SM-C receptor concentration and affinity.

The fibroblasts were plated and grown in Dulbecco's MEM, as described above, in multiple 60-mm tissue culture dishes. The plates were trypsinized at intervals ranging in time from 2 to 13 days and counted in a Coulter counter. There was no qualitative difference of the fibroblast growth curve in this patient when compared to those of three normal adults and three patients with Turner's syndrome (Figure 1).

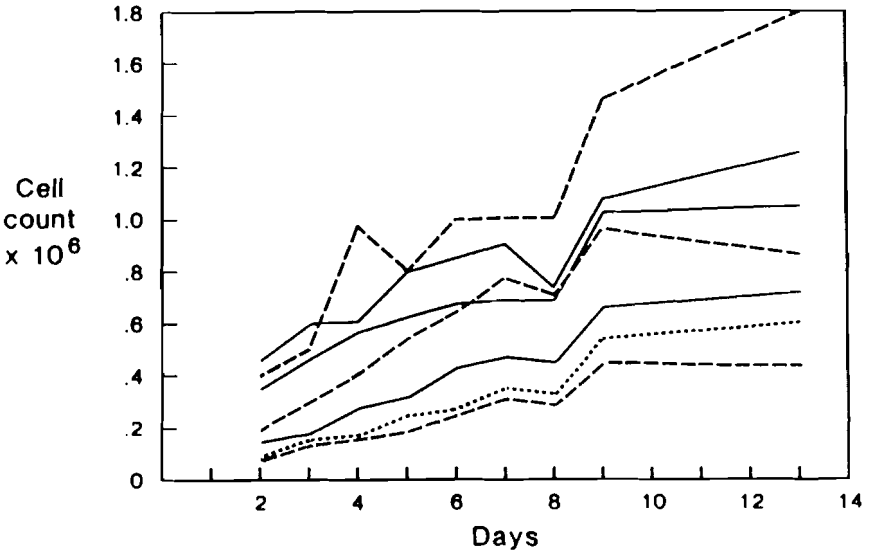

Figure 1. Fibroblast growth curves. Fibroblasts from patient 1 (*dotted line*), three normal adults (*dashed lines*), and three patients with Turner's syndrome (*solid lines*) were plated on day 0 in multiple tissue culture dishes. The plates were trypsinized at intervals ranging in time from 2 to 13 days and the cells were counted by Coulter counter.

Serum and plasma protein dialysates were prepared from both patients. The ammonium sulfate precipitation used was that of Pederson (1947). Serum or plasma was diluted with an equal volume of 0.2 M sodium chloride, followed by addition of ammonium sulfate until a concentration of 45% $(NH_4)_2SO_4$ was obtained. The precipitate was centrifuged at 17,700 × *g* for 45 minutes, after which the supernatant was decanted. The pellet was washed twice by resuspension in 2.0 M ammonium sulfate and recovered by centrifugation as described above. The pellet was dissolved in 1 M acetic acid and dialyzed against 1% acetic acid in Spectropor 3 dialysis (molecular weight cutoff approximately 3,500; Spectrum Medical Industries No. 132720). Agar plates were prepared in 35-mm petri dishes by first applying a 1-ml base layer of 0.8% purified agarose in McCoy's 5A medium containing 10% FBS. After solidification, an additional 1 ml of 0.4% agarose in the same medium, but also containing the desired protein concentration of serum or plasma and 7,500 AKR-2B mouse fibroblasts, or the same number of normal rat kidney (NRK) fibroblasts, was applied to the upper layer. The dishes were incubated at 37°C in 5% CO_2 for 7 to 14 days, at which time the number of colonies per dish were counted by a computerized image analysis system.

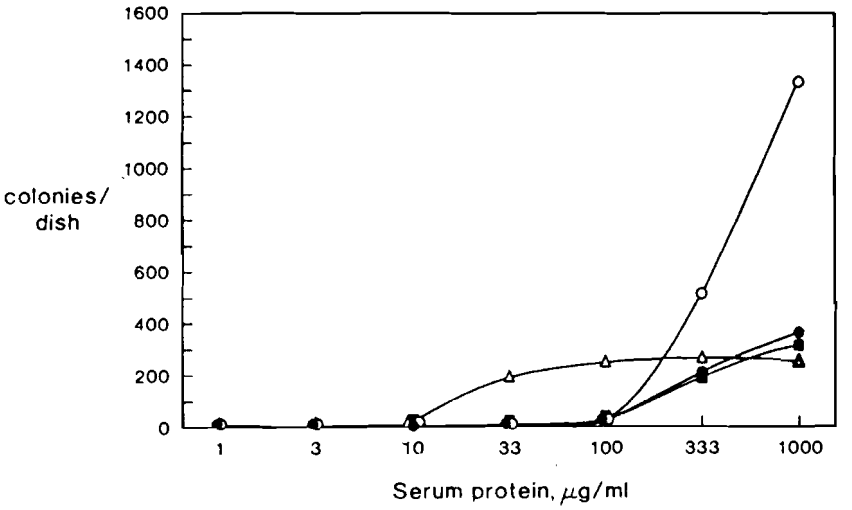

Figure 2. AKR-2B growth stimulation (patient 1). Stimulation of growth of AKR-2B mouse fibroblasts at various serum protein concentrations is illustrated for patient 1 (*open triangles*), a 19-month-old age- and sex-matched control (*open circles*), and one adult control assayed twice (*closed circles and squares*).

The serum protein from patient 1, in the concentration range of 10 to 100 μg/ml, significantly stimulated the growth of AKR-2B mouse fibroblasts as compared to a 19-month-old age- and sex-matched control and to one adult control assayed twice (Figure 2). The enhancement of growth at higher protein concentrations (333 and 1,000 μg/ml) was below that of the age-matched control, and comparable to the adult control.

To see if these results were reproducible, the same experiments were repeated for patient 1 using both serum and plasma protein dialysates. When compared to the same age- and sex-matched control, the same results, although more evident, were obtained (Figure 3). When serum and protein dialysates from patient 2 were assayed in a similar manner, the results did not differ from those of a 10-month-old age- and sex-matched control (Figure 4). When NRK fibroblasts were substituted from the AKR-2B cells, the results were irreproducible.

DISCUSSION

In our study of two patients with cerebral gigantism, we were able to confirm the results of previous investigators that the serum levels of growth hormone and SM-C are unremarkable. NGF and EGF serum

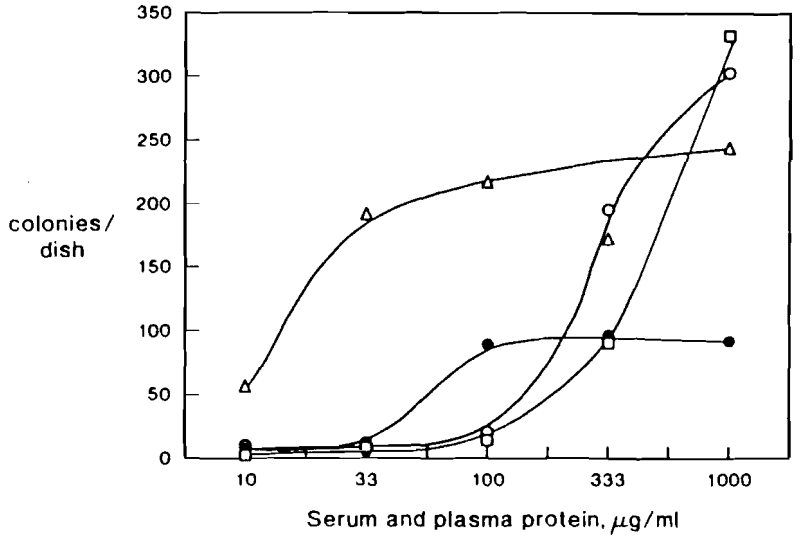

Figure 3. AKR-2B growth stimulation (patient 1). Stimulation of growth of AKR-2B mouse fibroblasts at various serum and plasma protein concentrations is illustrated for patient 1 (serum, *open triangles*; plasma, *closed circles*) and for a 19-month-old age- and sex-matched control (serum, *open circles*; plasma, *open squares*).

levels in patients with this syndrome had not been previously investigated, and were found to be comparable to those of normal adults.

In order to investigate the possibility of enhanced tissue growth responsiveness as reflected in surface growth factor receptor concentrations, a skin biopsy was obtained from patient 1 and fibroblast cultures established. When grown in confluent monolayer culture, the fibroblasts displayed EGF and SM-C binding that did not differ significantly from the controls. Furthermore, qualitative growth kinetics of these fibroblasts were not distinguishable from those of normal adults and patients with Turner's syndrome (Figure 1). These fibroblast culture experiments had not been previously performed in patients with cerebral gigantism.

In patient 1, serum and plasma protein dialysate, in the concentration range of 10 to 100 μg/ml, significantly stimulated the growth of AKR-2B mouse fibroblasts in tissue culture when compared to adult and age-and sex-matched controls (Figure 2). These results were reproducible for both patients as well as for the age-and sex-matched and adult controls, as illustrated in Figures 2, 3, and 4. This technique, although used to assay transforming growth factors as described by Moses et al. (1981), has not been previously applied to the study of

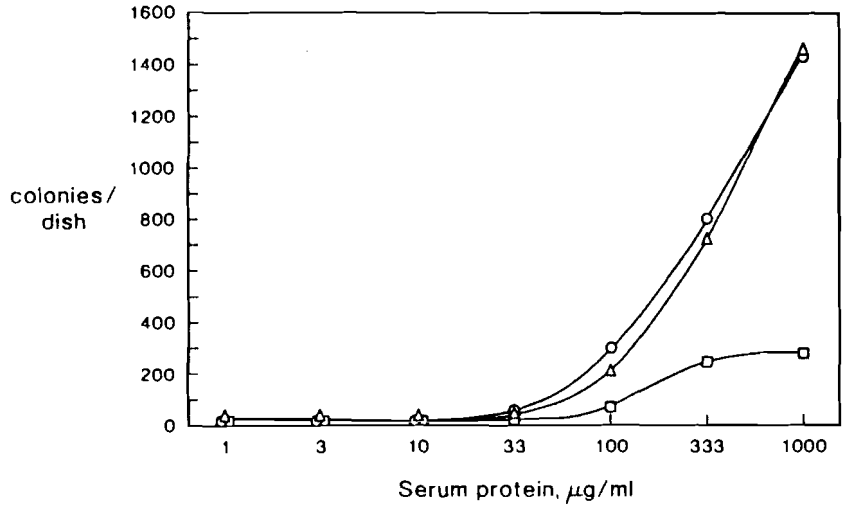

Figure 4. AKR-2B growth stimulation (patient 2). Stimulation of growth of AKR-2B mouse fibroblasts at various serum protein concentrations is illustrated for patient 2 (*open triangles*), a 10-month-old age- and sex-matched control (*open circles*), and an adult control (*open squares*).

patients with cerebral gigantism, nor to any other disorders of growth and mentation.

The serum and plasma protein stimulation of AKR-2B mouse fibroblast growth in one child may be a demonstration of a novel growth factor in cerebral gigantism. The reason why one patient demonstrated such a serum and plasma property and the other did not may be due to an age-related effect (patient 1 was 19 months old and patient 2 was 10 months). Alternatively, cerebral gigantism may be a heterogeneous disease with different causes. This syndrome has been associated with a variety of disease processes including thyroid abnormalities, cerebral dysgenesis, macular degeneration, autonomic insufficiency, peripheral dysostosis, and intestinal polyposis. Recently, Plioplys and Gomez (in preparation) noted markedly variable cranial computed tomography findings in patients with cerebral gigantism, as well as increased spinal fluid pressure in two children with this condition. All of these diverse associations argue that this syndrome may arise from various causes, one of which may be related to this demonstrable growth effect.

Further investigations using the AKR-2B mouse fibroblast assay technique are warranted in cerebral gigantism and may have applicability to the study of the possible causes of abnormal growth and mentation in other syndromes.

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