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End-organ resistance to growth hormone and IGF-I in epiphyseal chondrocytes of rats with chronic renal failure

ROBERT H.K. MAK and YOUNGMI K. PAK

Division of Nephrology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA

End-organ resistance to growth hormone and IGF-I in epiphyseal chondrocytes of rats with chronic renal failure. We tested the hypothesis that there is direct end-organ resistance to growth hormone (GH) and IGF-I in chronic renal failure (CRF) independent of circulating inhibitors. Male Sprague-Dawley rats underwent 5/6 nephrectomy and were pair-fed with weight matched (100 g) sham operated controls for two weeks. Rats with CRF had significantly higher serum creatinine and blood urea nitrogen ($P < 0.01$ in both cases) and gained significantly less weight and length ($P < 0.01$ in both cases) compared with controls. Epiphyseal chondrocytes were grown in 10% fetal calf serum (FCS). Both CRF cells and control cells maintained chondrogenic phenotypes, and showed immunohistochemical staining with antibodies to collagen II and proteoglycan (aggrecan). Distribution of the cell subpopulations according to cell size (by flow cytometry) and alkaline phosphatase activity of CRF and control chondrocyte cultures were not different. Growth responses of CRF chondrocytes were reduced ($P < 0.01$) compared with control chondrocytes when grown in 10% FCS and 10% normal rat serum. Under serum free conditions, growth responses of CRF chondrocytes were reduced to GH and IGF-I at concentrations of 10, 30 and 100 ng/ml, and to insulin at 100, 300 and 1,000 ng/ml compared with controls cells ($P < 0.01$). To show that this resistance is specific for the GH/IGF system, growth responses to fibroblast growth factor and transforming growth factor $\beta 1$ were studied and showed no difference between CRF and control cells. Thus, the present study provides direct evidence of specific end-organ resistance to GH, IGF-I in CRF chondrocytes in the absence of circulating factors.

Growth retardation is a serious consequence of chronic renal failure (CRF) in childhood. The pathogenesis is clearly mutifactorial including nutritional, electrolyte and hormonal disturbances. Attempts at correcting nutritional and electrolyte disturbances have failed to normalize growth in CRF [1]. A recent report from the North American Pediatric Renal Transplant Cooperative study showed that the mean baseline height of over a thousand children with CRF was below two SD scores at the time of transplantation [2]. This indicates that growth retardation continues to be an unresolved consequence of CRF despite optimal clinical management.

Circulating levels of GH and IGF-I are normal in children with CRF despite growth retardation suggesting resistance to these growth factors. Current evidence suggests that resistance to these growth factors in CRF are due to circulating inhibitors and that these inhibitors may be in the form of binding proteins (BP). Increased GHBP has been shown in CRF rats and may be involved in the pathogenesis of GH resistance in CRF by competing for ligand binding to the GH receptor [3]. Increased serum IGF binding capacity as well as increased serum IGFBP-1 and IGFBP-3 are present in children with CRF [4]. Removal of these excessive IGFBPs from CRF sera by affinity chromatography resulted in a significant increase in IGF bioavailability by cartilage explant sulfate incorporation. IGF resistance in CRF may therefore be due to the presence of excess circulating IGFBPs [4, 5]. However, Martin et al showed that supraphysiological doses of IGF-I and des-IGF-I (an analogue with reduced affinity for the IGFBPs) can equally correct growth retardation in rats with CRF, suggesting that there may be end-organ resistance to IGF-I independent of circulating IGFBPs [6].

The present study tests the hypothesis that there is direct end organ resistance to GH and IGF-I independent of circulating inhibitors in a rat model of CRF. We use a primary culture of epiphyseal chondroyctes to study growth stimulating effects of individual growth factors in the absence of other serum factors on the cells that account for longitudinal growth.

Methods

Experimental model

Male Sprague-Dawley rats weighing between 85 to 90 g were purchased from Charles River Laboratories (Wilmington, MA, USA) and housed in an environmentally controlled room with a 12 hour light/dark cycle, where they had free access to standard rat chow (Wayne MRH 22/5 Rodent Blox 8640) containing 22.2% protein and 0.36% sodium. After three days of equilibration in the new environment, CRF was induced in the animals by 5/6 nephrectomy in a one stage procedure. Under anesthesia (intraperitoneal pentobarbital 50 mg/kg) approximately two-thirds of one kidney was removed through a flank incision. When hemostasis was achieved, the contralateral kidney was then removed in through another flank incision under the same anesthesia in a one stage procedure. Each control animal was matched for weight with an uremic animal and underwent the same anesthesia and sham operation (decapsulation of the kidneys) a day later. All animals were housed in individual cages and were pair-fed. The CRF rats were fed *ad libitum*. Daily food intake of CRF rats was measured and the same amount of food was given to the allocated paired sham control the following day. The total period of observation was two weeks, at the end of which the animals were sacrificed by an overdose of sodium pentobarbital. The snout to tail lengths were measured at the time of nephrectomy and

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sacrifice, in both instances under anesthesia so that the animals were totally relaxed. Blood was taken for measurement of serum creatinine and blood urea nitrogen. Both knee joints were dissected out under sterile conditions. All data points were derived from six independent experiments. Each data point in these independent experiments was derived from cells from two rats (either 2 control rats or 2 CRF rats). The total number of rats in six independent experiments were 24 (12 control rats and 12 CRF rats).

Primary epiphyseal chondrocyte culture

Cartilage from rat tibial growth plate was dissected under sterile conditions and placed in a petri dish with Dulbecco's modified Eagles medium (DMEM) with 50 μg/ml gentamicin and minced with a scalpel. Bacterial collagenase (Type II and IV, Worthington Biochemicals, Freewood, NJ, USA) were added a final concentration of 0.6 mg/ml and the mixture was incubated overnight at 37°C in 7.5% CO_2 and 100% humidity. The cells were then collected, washed with phosphate buffered saline (PBS) and filtered though a 100 μm polypropylene mesh. Cell number was determined by counting on a hemocytometer and viability was determined by tryphan blue dye exclusion. In each isolation, cells from two rats per group were pooled with a cell yield of 3 to 5 $\times$ 10^5 cells per rat and viability was over 90%. This cell yield was similar to the one reported previously by Ohlsson et al [7]. Subsequently, cells were suspended in complete media: 1:1 mixture of DMEM and Ham's F12 supplemented with gentamicin and either 10% control rat serum or 10% fetal calf serum (FCS). First, the cells were seeded in 24 well plates at a density of 4 $\times$ 10^4 cells per well and growth responses (MTT, thymidine incorporation and cell number) were assessed after 72 hours of incubation with the complete medium containing 10% control rat serum. Then, 5 $\times$ 10^5 cells were seeded in 100 mm culture dishes and cell number was assessed after seven days of incubation with the complete medium containing 10% FCS. Culture media were changed every three days in the latter experiment.

For studies relating to growth responses to different growth peptides the cells were seeded in 24 well plates coated with poly-L-lysine at a density of 4 $\times$ 10^4 cells per well and allowed to attach to the plate in 10% FCS for 24 hours. Then they were incubated with serum free medium (SFM), which is a 1:1 mixture of DMEM and Ham's F12 supplemented with gentamicin, 20 nM selenium and lipid emulsion containing lecithin, cholesterol, sphingomyelin, vitamin E and vitamin E acetate, for 48 hours before the test peptides (human recombinant IGF-I, Genentech Inc, San Francisco, CA; human recombinant insulin, Eli Lily Inc, Indianapolis, IN, USA) in different concentrations were added into each well and further incubated for 72 hours. The incubation period with the test peptide was increased to 96 hours for studies relating to GH (human recombinant GH; Genentech Inc.) growth responses. For growth assessment, cell number, ^{3}H-thymidine incorporation and mitogenic activity using methylthiazol tetrazolium (MTT) [8] were determined.

Immunohistochemistry

Chondrocytes were cultured in complete medium with 10% FCS on sterile glass cover slips until 80 to 90% confluent. They were then fixed in 1:1 mixture of acetone/methanol for 10 minutes and then stained with rabbit antibovine collagen type II and rabbit anti-bovine proteoglycan (aggrecan) antiserum (generous gift of Dr. R.L. Smith, Functional Restoration, Stanford University). Goat anti-rabbit IgG (H+L) conjugated with alkaline phosphatase (Bio-Rad, CA, USA) was used as a second antibody and developed with nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) substrates.

Flow cytometry

Cells were trypinized, counted, washed and resuspended in complete media with 10% FCS. Flow cytometry was used to measure distribution of cell subpopulations according to cell size. Forward light scatter was measured on a FACSTAR flow cytometer (Becton-Dickinson).

Alkaline phosphatase activity

A soluble protein extract of cells was obtained using 0.1% Triton X-100 (10 mM Tris-HCl, pH 7.4) for 60 minutes at 4°C. Enzyme activity was assayed in 100 μl aliquots by measuring the release of ρ-nitrophenol from the substrate ρ-nitrophenyl phosphate disodium (Sigma: Technical Bulletin 104) and then assessed by spectrophotometry at 405 nm. Protein content of the CRF and the control chondroyctes was measured by the Bio-Rad DC protein assay and the alkaline phosphatase activity was expressed as μmol/min/mg protein.

Methyl thiazol tetrazolium (MTT) mitogenic activity assay

Chondrocytes were seeded on 96 well plates at the density of 10,000 cells per well. The desired concentrations of reagents were added in each well and incubated for 72 hours at 37°C, 7.5% CO_2. The assay mixture containing C3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added into each well and incubated under the same conditions for four hours. Purple precipitates generated by live cells were extracted with HCl/isopropanol and the optical density of each well was determined using ELISA plate readers (Bio-Rad) at 570 nm [8].

^{3}H-thymidine incorporation

Chondrocytes grown on 24 well plates were labeled with 5 μCi/ml ^{3}H-thymidine (New England Nuclear, MA, USA) per well for 18 hours before terminating the reaction. The media were removed and the cells were washed with PBS. DNA was precipitated with 10% TCA at 4°C, redissolved in NaOH and the incorporated radioactivity was counted.

Statistics

All data points were derived from six independent experiments. Each data point in these independent experiments was derived from cells from two rats (either 2 control rats or 2 CRF rats). The total number of rats in six independent experiments were 24 (12 control rats and 12 CRF rats). The data were tested for normality by the chi square test and then analyzed for statistical difference using the unpaired *t*-test. Bonferroni correction was made for multiple *t*-tests when different methods were used to measure growth responses. Analysis of variance with the Student-Newman-Keuls test was performed for comparing growth responses to different concentrations of peptides.

Results

The weights and lengths as well as the dietary intakes in the control and CRF rats are presented in Table 1. Rats with CRF and control rats were not different in initial weights and lengths.

Table 1. Growth, serum chemistry and food intake of the CRF and control rats

Group	Initial weight	Weight gain	Initial length	Length gain	Serum creatinine	BUN	Food intake	Caloric intake
	g		*cm*		*mg/dl*		*g*	*Kcal*
CRF ($N = 12$)	99.1 ± 2.1	32.2[a] ± 7.1	27.4 ± 0.5	4.3[a] ± 0.4	0.7[a] ± 0.1	35.8[a] ± 4.4	141 ± 6	563 ± 24
Sham ($N = 12$)	99.7 ± 2.2	57.5 ± 4.3	27.0 ± 0.3	6.3 ± 0.7	0.4 ± 0.1	12.5 ± 1.3	141 ± 6	563 ± 24

Data are mean ± SD. The serum creatinine and BUN measurements were made at the end of the study.
[a] $P < 0.01$

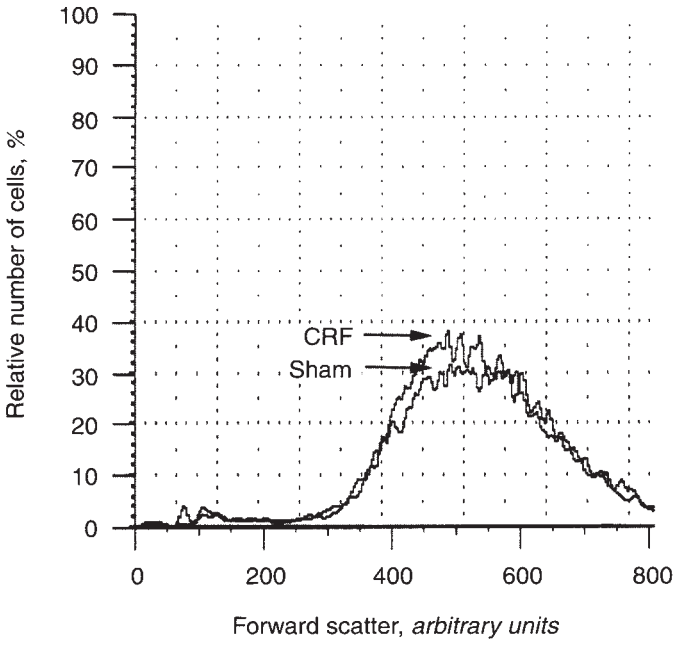

Fig. 1. *Forward scatters of the CRF and control chondrocyte cultures as measured on a FACSTAR flow cytometer.*

Over the two-week experimental period, rats with CRF consumed equal amounts of food as compared with the controls but gained significantly less weight (56% of control values, $P < 0.01$) and length (68% of control values, $P < 0.01$). The rats with CRF also had higher serum creatinine and blood urea nitrogen ($P < 0.01$ in both cases). The degree of renal insufficiency in the rats in the present study is only modest compared with other studies using 5/6 nephrectomy protocols [9]. Nonetheless, the CRF rats were growth retarded compared with sham-operated pair-fed controls.

To demonstrate that both CRF and control epiphyseal chondrocytes maintain their chondrogenic phenotype in primary culture, immunohistolochemical staining of the cells grown on cover slides was performed with antibodies to proteoglycan and collagen II revealing positive results in both CRF and control cell cultures. To investigate the possibility that the primary cultures of CRF and control chondrocytes contain different subpopulations of cells, distribution of the subpopulations according to cell size was studied by flow cytometry. The forward scatters of CRF and control chondrocytes are almost identical (Fig. 1). Also the alkaline phosphatase activity of the two cell populations was measured and there was no difference between CRF cells (6.13 ± 0.14 μmol/min/mg protein) and control cells (5.87 ± 0.59 μmol/min/mg protein).

The growth responses of CRF and control chondrocytes to different concentrations of normal rat serum were then studied using the MTT test. Figures 2A shows that the growth responses of CRF chondrocytes are significantly reduced ($P < 0.05$) compared with control chondrocytes at 10% concentration of normal rat serum. The growth responses were then measured again, by thymidine incorporation and cell number. The results are shown in Figure 2 B (thymidine incorporation) and C (cell number), indicating that the growth responses of the CRF chondrocytes to 10% normal rat serum are reduced compared with control chondroyctes ($P < 0.05$ in both cases; Bonferroni correction was performed for multiple t-tests). Similarly, growth (cell number) of CRF chondrocytes in the presence of 10% FCS is also much reduced compared with control chondroyctes ($P < 0.01$, Fig. 3).

The growth responses of the chondrocytes to various growth factors in serum free media were studied and the results are shown in Figure 4. Growth responses of CRF chondrocytes to GH in serum free media at 1, 10, 30 and 100 ng/ml concentrations are reduced compared with control chondrocytes ($P < 0.01$ in all 3 cases) indicating growth hormone resistance. Growth responses of CRF chondrocytes to IGF-I in serum free media are also reduced at 1, 10, 30 and 100 ng/ml concentrations compared with control chondrocytes ($P < 0.01$) indicating IGF-I resistance. At low concentrations of insulin at 1 and 10 ng/ml (which did not significantly stimulate growth in control chondrocytes), there are no differences in the growth responses between the two different cell types. However, growth responses of CRF chondrocytes to insulin in serum free media at 100, 300 and 1,000 ng/ml are reduced compared with control chondrocytes ($P < 0.01$). Since these insulin concentrations are supraphysiological and are more likely to involve the IGF type I receptor, this provides further evidence of IGF-I resistance. To demonstrate that the resistance to growth stimulation in CRF chondrocytes is specific for the GH/IGF system, growth responses to fibroblast growth factor (FGF) and transforming growth factor (TGF) β1 were studied. There are no significance differences between CRF and control chondrocytes in terms of their growth responses to FGF and TGF β1 over a range of concentrations shown in preliminary studies to effectively stimulate normal chondrocyte growth. These results are shown in Figure 5. Finally, these chondrocytes only maintained their impaired responsiveness to serum and growth factors for two passages. This phenotype was not present after one month of culture.

Discussion

A critical function of the epiphyseal chondrocytes is the production of cartilage matrix, primarily composed of cartilage collagens and proteoglycans. Type II collagen is the predominant structural collagen of cartilage and the distinguishing collagen

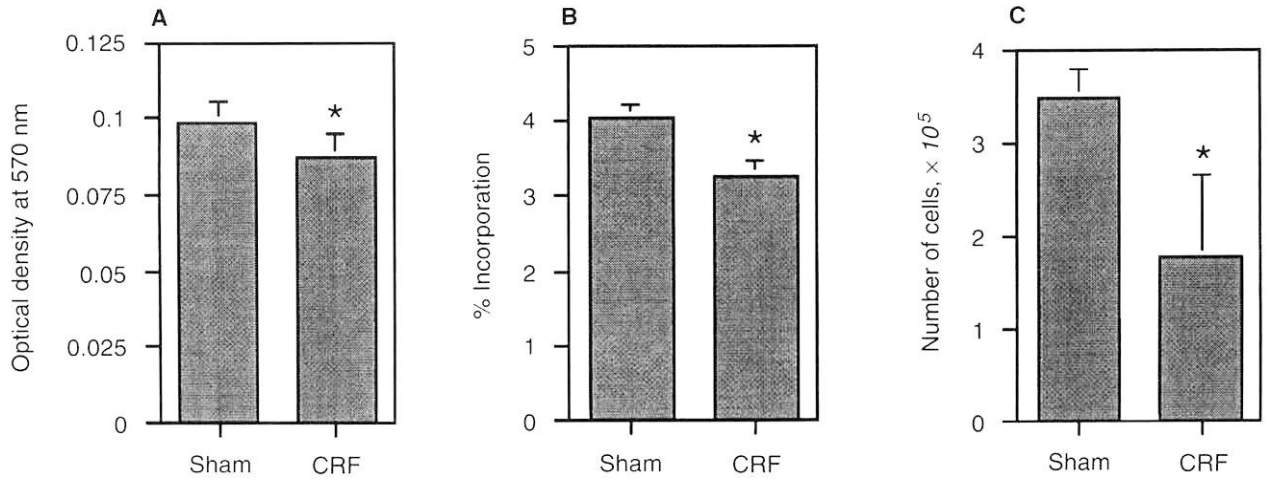

Fig. 2. *Effect of 10% control rat serum on growth over a three days period of chondrocytes from CRF and control rats as measured by the MTT test* (**A**), *^{3}H-thymidine incorporation* (**B**) *and cell number* (**C**). $N = 6$ independent experiments, that is, 12 rats per group. Data are mean ± SD; $^*P < 0.05$ by unpaired t-test with Bonferroni adjustment; CRF vs. control.

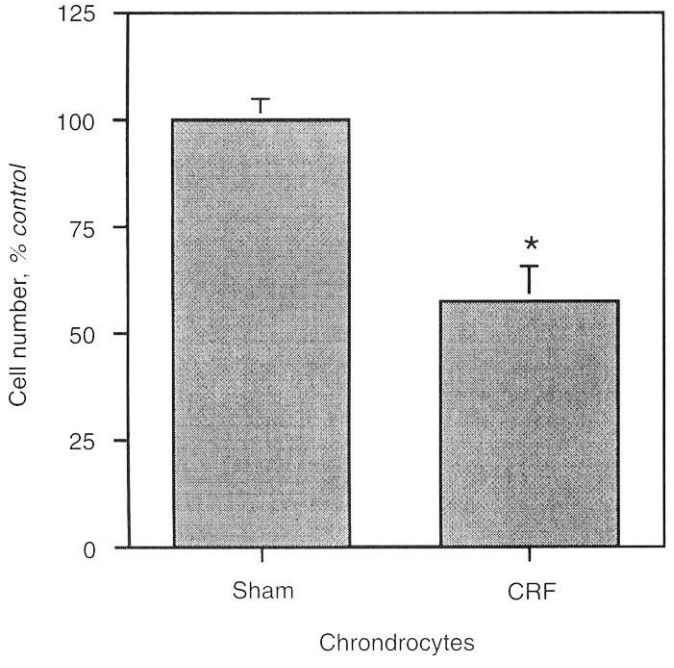

Fig. 3. *Effect of 10% FCS on growth over a seven days period of chondrocytes from CRF and control rats as measured by cell number.* $N = 6$ independent experiments, that is, 12 rats per group. Data are mean ± SD; $^*P < 0.01$ by unpaired t-test, CRF vs. control.

type synthesized by chondrocytes [10]. Studies employing histochemical methods have suggested that type II collagen is homogenously distributed throughout the growth plate [11]. The characteristic proteoglycan produced by chondroyctes is aggrecan, a large aggregating proteoglycan substituted with up to 100 chondroitin sulfate chains. The synthesis of type II collagen coupled with the synthesis of aggrecan traditionally defines the chondrocyte phenotype [8]. In the present study, the demonstration of both collagen II and aggrecan by immunohistochemistry in epiphyseal chondrocytes from both CRF and control rats grown in monolayers indicate that these cells are differentiated chondrocytes. This is in agreement with previous studies by Ohlsson et al demonstrating that rat epiphyseal chondrocytes in primary monolayer cultures maintain their chondrogenic phenotype up to 15 days [7].

In the normal growth plate, chondrocytes undergo a series of developmental changes in structure and function through the resting, proliferative, maturing and hypertrophic zones before calcification occurs. Alini et al [12] showed that bovine fetal epiphyseal chondrocytes can be divided into five subpopulations according to cell density and size (from the smallest resting cells to the largest hypertrophic cells). Alkaline phosphatase activity correlates with cell size in these bovine fetal chondrocytes [13]. There are no corresponding data in rats to date. *In vitro* studies using mixtures of growth plates chondrocytes have produced conflicting results as to how these cells respond to different growth factors and hormones. This is probably because the responses of chondrocytes from different regions of the growth plates are dependent upon their maturational state. Also, mixed cultures may contain varying proportions of different cells in different maturational and responsive states. The growth plate in CRF rats is altered [9]. It is therefore possible that the differences in growth responses of the CRF chondrocytes is not related to the renal insufficiency but to the selection of different chondrocyte subpopulations. However, the distribution of the cell subpopulations according to cell size in CRF and control chondrocyte cultures as studied by flow cytometry were almost identical. Also the alkaline phosphatase activity was not difference between CRF cells and control cells. Thus differences in different chondrocyte subpopulations are not the likely cause of growth response differences between CRF and control epiphyseal chondrocyte cultures in the present study.

In 10% normal rat serum, the growth responses of CRF chondrocytes are significantly reduced compared with control chondrocytes. These growth responses have been measured by three different methods which yield the same results. Furthermore, this phenomenon is confirmed by the growth responses of the chondrocytes in 10% FCS. This is in agreement with the clinical data that the rats with CRF are growth retarded compared

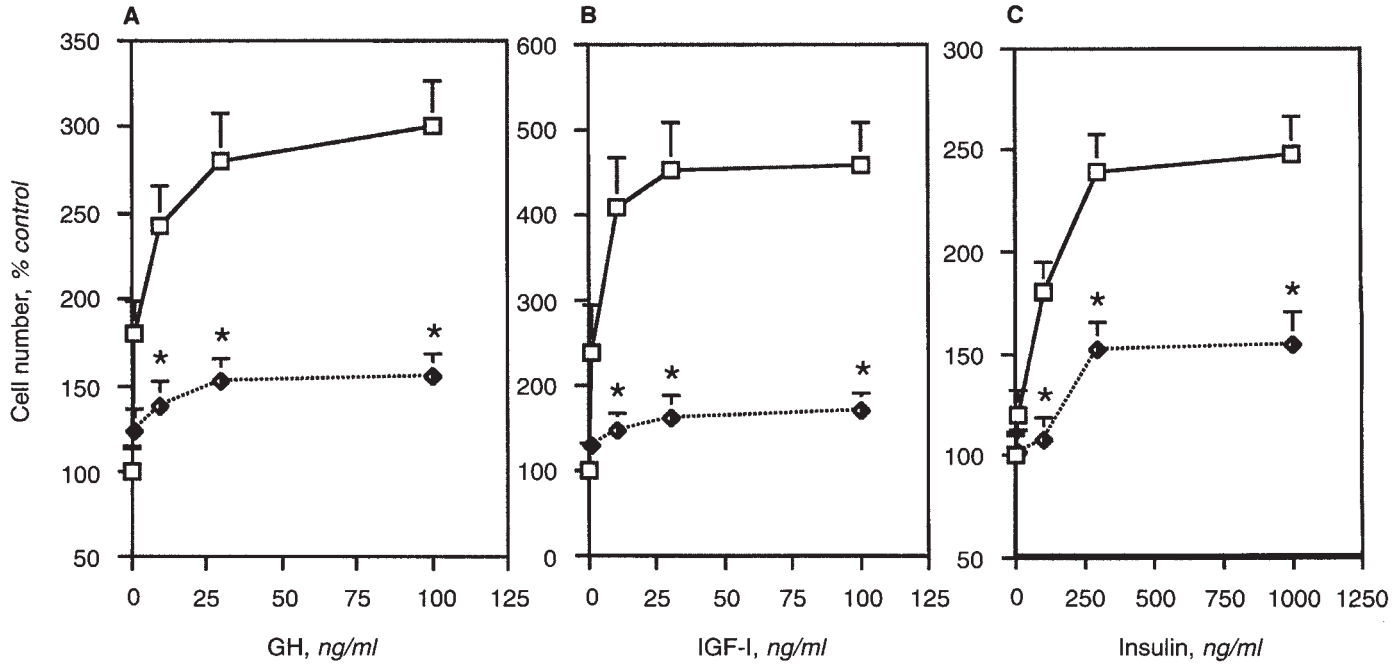

Fig. 4. *Effect of (A) GH, (B) IGF-I and (C) insulin on the growth of chondrocytes from CRF (··◆··) and control (-□-) rats.* The primary cells from each group were seeded on 24-well plates at a density of 4×10^4 and grown in the presence of complete media to allow the cells to attach to the plate. Then the cells were serum-starved for 48 hours. Different concentrations of GH were added to each well and incubated with the cells for 96 hours. Different concentrations of IGF-I and insulin were added to each well and incubated with the cells for 72 hours. The cells were then harvested by trypsinization and the cell numbers were determined with a hemocytometer. $N = 6$ independent experiments, that is 12 rats per group, data = mean ± SD. Growth responses of CRF chondrocytes to GH at 1, 10, 30 and 100 ng/ml concentrations are reduced compared with control chondrocytes ($P < 0.01$ in all cases). Growth responses of CRF chondrocytes to IGF-I in serum free media are also reduced at 1, 10, 30 and 100 ng/ml concentrations compared with control chondrocytes ($P < 0.01$ in all cases). At low concentrations of insulin at 1 and 10 ng/ml, there are no differences in the growth responses between the two different cell types. However, growth responses of CRF chondrocytes to insulin in serum free media at 100, 300 and 1,000 ng/ml are reduced compared with control chondrocytes ($P < 0.01$). *$P < 0.01$ by analysis of variance followed by the Student-Newman-Keuls test at all higher concentrations, CRF versus control.

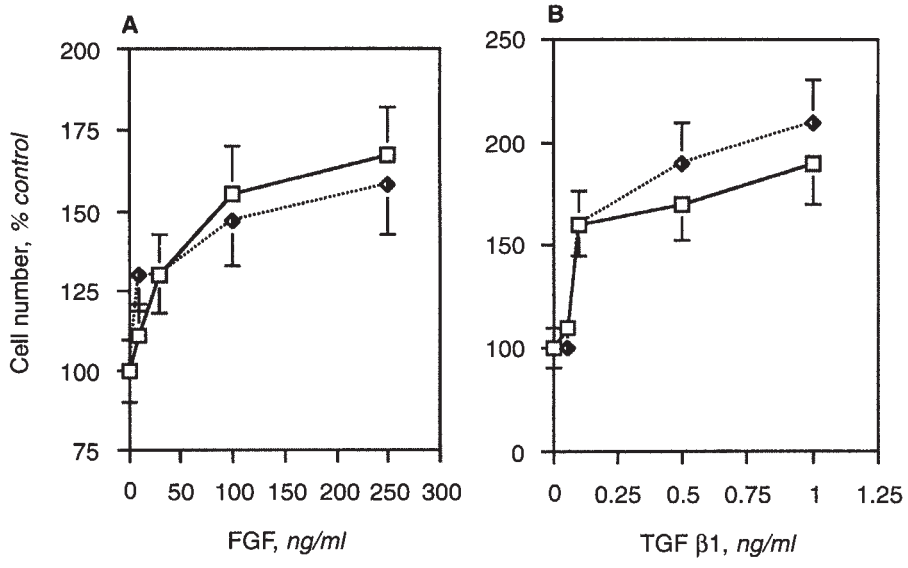

Fig. 5. *Effect of (A) FGF and (B) TGF β1 on the growth of chondrocytes from (··◆··) CRF and (-□-) control rats.* The primary cells from each group were seeded on 24-well plates at a density of 4×10^4 and grown in the presence of complete media to allow the cells to attach to the plate. Then the cells were serum-starved for 48 hours. Different concentrations of FGF (10, 30, 100 and 250 ng/ml) and TGF β1 (0.01, 0.1, 0.5 & 1 ng/ml) were added to each well and incubated with the cells for 72 hours. The cells were then harvested by trypsinization and the cell numbers were determined with a hemocytometer. $N = 6$ independent experiments, that is, 12 rats per group. Data are mean ± SD. No significant difference was found at all concentrations by analysis of variance followed by Student-Newman-Keuls test, CRF versus control.

with the pair-fed sham operated controls. Thus the chondrocytes from rats with CRF maintain the phenotype of growth retardation in primary culture.

Longitudinal bone growth is the result of recruitment of cells from the stem cell layer and subsequent proliferation of the differentiating cells in the growth plate of the long bones. Growth hormone (GH) stimulates longitudinal bone growth in a dose-dependent manner [14]. GH receptors have been demonstrated in rat epiphyseal chondrocyte cultures [15]. However, the stimulatory effect of GH is dependent on the presence of IGF-I [16].

GH-dependent IGF-I peptide immunoreactivity has been shown in the growth plates of hypophysectomized rats [17]. Insulin, in high concentrations, can compete for binding to the IGF type I receptor [18] and promotes proliferation in primary chondrocyte culture [19, 20]. Ohlsson et al [7] have previously shown that the time course for the stimulatory effect of GH and IGF-I on proliferation of epiphyseal chondrocytes grown in primary monolayer cultures are different. GH effects become significant after three days of culture whereas IGF-I effects are apparent during the first 24 hours of culture. Similar observation was obtained in our laboratory. Therefore the GH dose responses in the present study were performed with a longer incubation period with the test peptide in the present study. The concentrations of GH and IGF-I used in the dose response studies are within physiological range under serum free conditions in chondrocyte culture systems [7]. The maximum stimulatory effects of GH (~300%) and IGF-I (~450%) on control chondrocytes are similar to those reported previously by Ohlsson et al [7]. The maximum stimulatory effects of GH and IGF-I on CRF chondrocytes are much reduced (~150%) compared with control chondrocytes, indicating end-organ resistance in the GH/IGF axis in CRF. The concentrations of insulin (100 to 1,000 ng/ml or 2400 to 24,000 μU/ml) used are 24 to 240 times higher than insulin concentrations (around 100 μU/ml) achieved in humans during hyperglycemic stimuli. These concentrations are not physiological but have been reported to be competitive for binding to the IGF type I receptor and to stimulate chondrocyte matrix formation and proliferation [19, 20]. Therefore the insulin effects observed in the present study are likely to be related to the IGF type I receptor and not the insulin receptor. Two other growth factors are known to stimulate proliferation of chondrocytes in culture, namely FGF and TGF β1 [21]. The demonstration of responsiveness of CRF chondrocytes to FGF and TGF β1 indicates that the end-organ resistance to growth stimulation in CRF is specific for the GH/IGF axis.

The present study provides, for the first time, direct evidence of end organ resistance of epiphyseal chondrocytes to GH, IGF-I and insulin in CRF. In all previous studies of GH and IGF-I effects on longitudinal growth in CRF [6, 9], these peptides were administered to whole animals and therefore the effects of these peptides on overcoming circulating inhibitors in CRF such as elevated GHBP and IGFBPs were likely mechanisms. Mehls et al [9] treated CRF rats with supraphysiological doses of growth hormone for 14 days and achieved partial correction of their growth retardation. However, this was not accompanied by an increase in circulating IGF-I. The authors concluded that this constituted evidence of *in vivo* growth hormone resistance. Tonshoff et al demonstrated elevated circulating GHBP in CRF rats and postulated that this may be an inhibitor of GH action in CRF by competing for ligand binding to the receptor [3]. IGF-I resistance in uremic children could also be due to increased serum concentration of IGFBPs despite normal serum IGF-I concentrations [22]. Treatment of uremic children with GH improved the ratio between the serum concentrations of IGF-I and IGFBP and normalized the somatomedin bioactivity (measured by growth cartilage bioassay) of the sera from these patients [22].

There is indirect evidence to support end-organ resistance to GH and IGF in CRF. Chan, Valerie and Chan demonstrated decreased induction of hepatic IGF-I mRNA by GH in CRF rats and that it was accompanied by decreased hepatic GH receptor mRNA. The authors postulated that the GH resistance in CRF rats was partially due to decreased hepatic GH receptor at the transcription level [23]. Finidori et al showed reduced growth hormone binding in the liver of CRF rats [24]. Tonshoff et al also demonstrated decreased hepatic GH receptor mRNA in CRF rats and postulated that it might contribute to GH resistance in CRF [3]. There were no measurements of GH receptor peptide expression in these studies. Also, there are no studies to date on GH receptor peptide or mRNA expression in growth plates in CRF. Martin et al [6] administered supraphysiological doses of IGF-I to CRF rats and showed improved growth. They also demonstrated the growth responses of CRF rats to des-IGF (an analogue of IGF-I with reduced affinity to IGFBPs) were similar to the responses to equivalent doses of IGF. This provides indirect evidence that there is end-organ resistance to IGF-I independent of circulating IGFBPs since the responses to des-IGF would be expected to be much enhanced if elevated IGFBPs was the main mechanism of growth retardation.

Insulin resistance and hyperinsulinemia are common in children with CRF [25] and may be important in the pathogenesis of growth retardation. Growth velocity SD scores in children with CRF have been shown to correlate with the insulin secretion during hyperglycemia [26]. McCaleb et al have partially purified and characterized a factor from uremic sera that inhibits insulin-mediated glucose disposal in normal rat adipocytes [27]. However, the insulin resistance observed in the present study is at supraphysiological dose ranges and is therefore likely to be at the IGF type I receptor level rather than at the insulin receptor level. Since insulin does not bind to IGFBPs at all, this provides further evidence of end organ resistance at the IGF type I receptor level independent of IGFBPs.

Thus the present study provides direct evidence of end organ resistance to GH, IGF-I and insulin of epiphyseal chondrocytes in CRF. The mechanism of resistance to GH and IGF-I shown in these *in vitro* studies in the absence of CRF serum indicates that circulating inhibitors are not involved. However, this does not exclude contributions from serum factors in the *in vivo* situation. The primary cell culture system described in the present study provides a good *in vitro* model to study underlying mechanisms involved in the growth retardation of CRF.

Acknowledgment

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Reprint requests to Robert H.K. Mak, M.D., Room G306, Department of Pediatrics, Stanford University Medical Center, 300 Pasteur Drive, Palo Alto, California 94305-5119, USA.

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