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Quantitative Histology of Cartilage Cell Columns in the Human Costochondral Junction: Findings in Newborn and Pediatric Subjects

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ABSTRACT. The mean number of cells per cartilage column and the proportion of hypertrophic and proliferative chondrocytes per column were determined in the costochondral junction in a population of normal subjects including 10 fetal-newborns and 15 subjects aged 0.3–16 y of age. Both the mean number of cells per column and the proportion of proliferative cells per column were significantly greater in the fetal-newborn population compared to the pediatric population (12.6 ± 1.0 (10) *versus* 8.4 ± 0.4 (15), $p < 0.001$ and 39.6 ± 6.9 (10) *versus* 24.4 ± 2.5 (15), $p = 0.025$, respectively) (mean ± sem [n]). The number of cells per column bore a significant negative relationship to subject age ($r = -0.52$, $p = 0.007$). Significant positive correlations were found between the mean number of cells per column and age-specific growth velocity both in males (length-height velocity = [(6.3) (mean number of cells) − 44.1], $r = 0.72$, $p = 0.02$) and in females (length-height velocity = [(3.4 (mean number of cells) − 14.4], $r = 0.77$, $p = 0.006$). These data will provide normative values against which abnormalities characteristic of the skeletal dysplasias can be compared. (*Pediatr Res* 25:202–204, 1989)

Abbreviations

CV, coefficient of variation

Orderly development of cartilage cell columns has long been an intriguing aspect of the biology of chondrocytes (1, 2). Past studies have generally been of a qualitative nature; quantitative information on the histology and development of cartilage columns in the human is scarce. Kember and Sissons (3) have documented chondrocyte cell number and the proportion of chondrocytes in proliferative-maturational stages in the human femur, but normative data have not been reported for the costochondral junction, a skeletal sampling site which is commonly used in the evaluation of skeletal dysplasias. The skeletal dysplasias are a heterogeneous group of disorders which result in disproportionate short stature and/or skeletal deformities. Recent work has indicated that each of the chondrodysplasias appears to be associated with consistent chondroosseous histopathology which can presumably be related to specific defects in chondrocyte maturational, metabolic, biosynthetic and degradative processes (4).

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To begin a quantitative histologic assessment of abnormalities which characterize the skeletal dysplasias, we have evaluated the range of variability in cartilage column formation in the normal fetus, newborn, and young human. Our results suggest that quantitative histomorphometry will be a useful tool for the study of cartilage development. Our data indicate that the number of cells per column and the proportion of proliferative cells vary with the stage of development. The number of cells per column also correlates with sex-matched growth velocity data. Definition of the histologic features of normal cartilage at this site thus provides useful normative data against which the spectrum of alterations present in the skeletal dysplasias can be compared.

MATERIALS AND METHODS

Fetal, newborn, and pediatric specimens of the costochondral junction were obtained at autopsy from subjects who were aborted, stillborn, died from accidents or nonskeletal-related diseases, or were undergoing cardiac surgery. Specimens are part of the International Skeletal Dysplasia Registry collection. Specimens were embedded in methacrylate, sectioned longitudinally at 2.5 μm, and stained with Stains All (Eastman Kodak Co., Rochester, NY) as previously described (4, 5). Stains All was used because it provided good staining of both chondrocytes and the cartilage matrix and septal regions separating the columns.

Mean age for subjects excluding fetal and newborns was 7.1 ± 1.2 ($n = 15$) (mean ± SEM). In the fetal-newborn group, subjects ranged in age from 15 wk to newborn (nine of the 10 specimens were from newborns).

Quantitative evaluation was performed by visual counting of multiple sequential microscopic fields of each specimen at a magnification of 400×. The lowest hypertrophied cell with an intact cell membrane was counted as the first cell in the column. Counts were made of the number of hypertrophic and nonhypertrophic cells in each column. No distinction was made between early and late hypertrophic cells. Columns with more than two staggered clusters of cells were not scored. Two clusters were scored as one column only when surrounding matrix staining clearly demarcated them from surrounding cells and there was continuity between clusters. Morphologic classification of chondrocytes followed that of Brighton (6) in which proliferative zone cells are flattened cells aligned in columns. The hypertrophic zone begins when cells become spherical and enlarged. As noted by others (3), distinction between early hypertrophic and late proliferative zone cells was somewhat subjective in some columns. However, repeated counts of specimens showed variability of only 0.9% in mean number of cells per column and 3.3% in the means of the proportion of cells in the hypertrophic and nonhypertrophic zones.

To evaluate biologic reproducibility, five ribs from one normal subject (a 37-wk fetus) were dissected selecting the costochondral

junction, embedded, sectioned, stained, and evaluated for the proportion of hypertrophic cells per column. Means ± SD per rib were: 86.9% ± 0.156 ($n = 20$; CV, 0.18%); 86.7% ± 0.19 ($n = 10$, CV, 0.22%); 89.2% ± 0.17 ($n = 10$, CV, 0.19%); 95.7% ± 0.105 ($n = 12$, CV, 0.11%), and 94.9% ± 0.089 ($n = 10$, CV, 0.09%). Specimens were not available for similar evaluation of variation in the older pediatric group.

Mean number of columns evaluated in the fetal-newborn group was 18.1 ± 4.2 and ranged from 5 to 51. Mean number of columns evaluated in the pediatric group was 79.3 ± 10.7 and ranged from 36 to 176. Length-height growth velocity data used were those published by Smith (7).

Statistical analysis used calculation of CV and Student's t test for independent groups (8). Relationships between variables were evaluated using Pearson's correlation coefficient (8). Data are means ± SEM unless otherwise noted.

RESULTS

Fetal-newborn subjects had a significantly greater number of cells per column (12.6 ± 1.0, $n = 10$) than older pediatric patients (8.4 ± 0.4, $n = 15$, $p < 0.001$) and also a significantly larger proportion of proliferative cells in columns (39.6% ± 6.9) than did the pediatric subjects (24.4 ± 2.5, $p = 0.025$).

When all subjects were evaluated, the mean number of cells per column showed a significant decrease with age ($r = 0.52$, $p = 0.007$) (Fig. 1). No significant relation was present between the percentage of proliferative cells per column and age (Fig. 2, $r = -0.22$, $p = 0.3$) or between the number of cells/column and the percentage of nonhypertrophic cells per column ($r = 0.02$, $p = 0.9$).

We have also related our histologic findings with published normal length-height growth velocity data. The mean number of cells per column bore a significant positive correlation with age-specific length-height velocity in both males (Fig. 3*A*, $r = 0.72$, $p = 0.018$) and females (Fig. 3*B*, $r = 0.77$, $p = 0.006$).

DISCUSSION

Data on the number of cells per cartilage column and the manner in which this number is regulated in the human are scarce. Kember and Sissons (3) studied the human femur in 12 subjects aged 0–14 y. Although they did not evaluate their data for a relationship between the number of cells per column and age, in their data the correlation was significant and showed a decrease with age (no. of cells/column = 60.57 − (2.12)(age), $r = -0.83$, $p = 0.001$). The y-intercept maximum value for the mean number of cells was 60.6, a value greater that that which our correlation shows for the rib (11.4). The rate of change with age was also steeper for the femur (−2.12) compared with the rib (−0.32). These differences may be related to a greater linear growth in the femur. The femur, a wt-bearing long bone, would also be exposed to mechanical and stress forces different than those acting on the rib.

Cell number and cell activity are the two important factors which together determine the total activity of a cell population. Evaluation of our chondrocyte data shows that there is a general decrease in the mean number of cells per column in the rib from birth through the growth years. Because growth necessitates great activity in cartilage formation and endochondral bone formation,

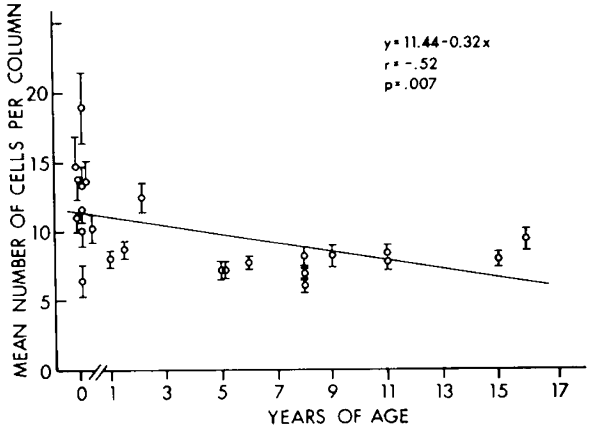

Fig. 1. Significant negative correlation between the mean number of cells per column and subject age ($r = -0.52$, $p = 0.007$). Each point is the mean ± SEM for an individual subject. Points positioned at 0 age represent fetal or newborn subjects.

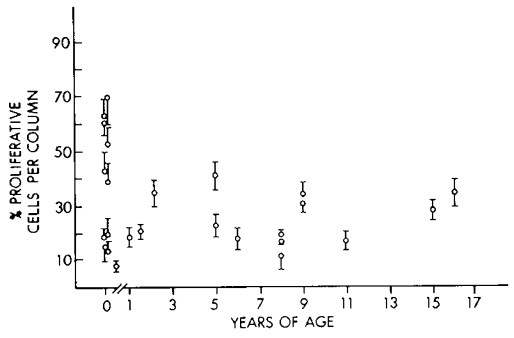

Fig. 2. Relationship between the proportion of proliferative cells and subject age. Data as in Figure 1.

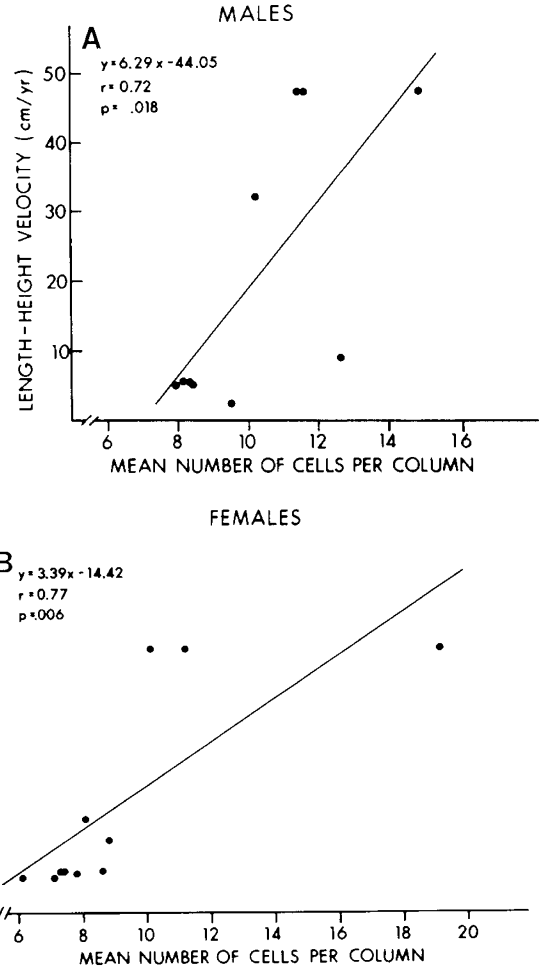

Fig. 3. Relationship between mean number of cells per column and age specific length-height velocity (6) in males (*A*, $r = 0.72$, $p = 0.02$) and females (*B*, $r = 0.77$, $p = 0.006$).

our data would suggest that changes in chondrocyte cell number (and possibly cell activity) are an important part of the orderly genetically programmed skeletal development.

Elucidation and clarification of the roles of regulatory peptides in the development of the skeletal system are important areas of current research. Studies by Nilsson *et al.* (9) of the proximal tibia of the rat have indicated that the IGF-I (somatomedin C) has a role in the clonal expansion of differentiated chondrocytes. They found that only chondrocytes in the proliferative zone contained IGF-I-like immunoreactivity. Growth hormone was found to increase the number of IGF-I reactive cells in the proliferative zone; it has been suggested that IGF-I stimulates clonal expansion of chondrocytes through autocrine/paracrine control. Nilsson *et al.* proposed that due to growth hormone stimulation, genes coding for growth factors of the somatomedin class are expressed during cell differentiation. This activation increases local production of IGF-I, which then promotes chondrocyte clonal expansion via autocrine or paracrine control (9). Another peptide which may figure in cartilage cell number regulation in the column is transforming growth factor-type β (identical with cartilage inducing factor A [10]). Transforming growth factor β has the ability to induce the cartilagenous phenotype and appears to have a morphogenetic action in skeletal regions that are involved in common congenital abnomalies, such as craniofacial structures (11). It is interesting, but at present merely speculative, to hypothesize that the increased numbers of cells we observed in younger infants might reflect the stimulatory effect of these factors. There are many important kinetic factors which our present work has not addressed. These include the rate of chondrocyte proliferation, the number of cells proliferating in the cartilage pool, the number of divisions before hypertrophy and the rate of the hypertrophic process. Vascular invasion and the calcification/ossification processes are additional other active factors which influence the dynamic structure of the growth plate.

Although chondrocyte cell number and proportions within columns are not always diagnostic or specific in themselves, we hope that increased quantitative histologic evaluation of the growth plate in normal and dysplastic conditions may provide data which can complement the unfolding information on the regulatory peptide control mechanisms acting on the developing and maturing skeletal system. In addition, it is hoped that such studies will add to our understanding of abnormalities present in achondroplasia, hypochondroplasia, and conditions with growth hormone deficiency or growth hormone resistance.

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