Development of the Cartilage Canals and the Secondary Center of Ossification in the Distal Chondroepiphysis of the Prenatal Human Femur

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The cartilaginous epiphysis of the distal femur is vascularized by a network of cartilage canals during prenatal development. The vascular invasion of the epiphysis begins at approximately eight to ten weeks of gestation with the initiation of cartilage canal formation. A complex vascular system develops within the canals and is well defined by fourteen weeks of gestation. The vascular system is fully developed several months prior to the development of the secondary center of ossification. The formation of the secondary center of ossification within the distal femoral epiphysis is preceded by changes that occur simultaneously within both the chondrocytes in the central portion of the epiphysis and the vascular and perivascular elements contained within the cartilage canals in the central portion of the epiphysis. These concurrent changes in the cellular morphology of the central chondrocytes and in the cellular structure of the central cartilage canals appear to be linked with the initiation of the process of osteogenesis.

INTRODUCTION

The process of ossification within the cartilaginous epiphysis of long bones is strikingly different from the pattern of ossification that occurs in the diaphysis. Cartilage canals are not formed in the diaphysis prior to the initiation of endochondral ossification in the primary center of ossification [4, 12, 13, 23, 26]. In the epiphyses, the cartilaginous anlagen is penetrated by a complex canal network which extends centrally from the surrounding perichondrium [14, 15, 17, 21, 22]. These cartilage canals undergo a well defined pattern of differentiation as they permeate the chondroepiphysis [11, 32]. The role of the cartilage canals in the initiation of ossification and the development of the secondary center of ossification has not been firmly established [1, 2, 6, 8, 10, 19, 20, 30, 31, 33]. This study details the temporal relationship between changes in the morphology of the central chondrocytes of the distal femoral epiphysis, the development of the cartilage canal network, and the formation of the secondary center of ossification.

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MATERIALS AND METHODS

Twenty pairs of prenatal human femurs ranging in age from six weeks to thirty-two weeks of gestation were studied; four femurs from full-term stillborns were also examined. The gestational age in the prenatal group was determined by measuring the crown-rump length of the fetus prior to dissection and removal of the femurs. The femurs were carefully dissected from the prenatal specimens; care was taken to keep the perichondrial attachments to the femoral epiphyses intact and undisturbed. All specimens were radiographed, photographed, and then decalcified in a 5% formic acid solution. Following decalcification, the specimens were embedded in paraffin or celloidin and serially sectioned at six to ten microns in either the frontal or sagittal plane. Each specimen was stained with hematoxylin and eosin and examined by microscopy. Special stains including saffranin-o and fast green were used on a selected basis.

RESULTS

Initial vascularization of the cartilaginous anlage

During normal endochondral ossification the entire femur initially forms a cartilaginous anlage that is avascular. There are no vessels or cartilage canals penetrating the densely packed chondrocytes from the surrounding perichondrium, although the peripheral tissues are very vascular. At six to eight weeks of development, the chondrocytes located within the central diaphyseal portion of the femoral anlage begin to hypertrophy. Eventually a primary center of ossification develops. Following the hypertrophy of the central chondrocytes, a circumferential bone collar (membranous ossification) and a central medullary canal (endochondral ossification) are rapidly formed. Primitive vascular channels extend through the bone collar into the newly formed medullary portion of the femoral diaphysis. Cartilage canals are not formed in the diaphyseal region prior to the development of the primary center of ossification. Endochondral ossification of the femoral diaphysis then extends proximally and distally as the central zone of ossification expands toward the epiphyses. Vascular channels and nutrient vessels develop from the periosteal blood vessels which cross through the bone collar and extend into the medullary canal of the femur.

Initial vascularization of the distal femoral epiphysis

The process of vascularization of the chondroepiphysis occurs through a process which is strikingly different from the adjacent diaphyseal region. Initially the chondroepiphyses are avascular and are composed exclusively of tightly packed chondrocytes until eight to ten weeks of gestation. Cartilage canals, originating as small collections of pluripotential mesenchymal cells arising from the surrounding perichondrium, protrude into the cartilaginous anlage. These focal invaginations of mesenchymal elements occur circumferentially in the region of the femoral condyles (Figure 1). No canals originate along the articular joint surface which does not have a perichondrium. Following the initial peripheral penetration of the chondroepiphysis, the mesenchymal cells extend from the perichondrium toward the central portion of the epiphysis. These mesenchymal tissue elements are characterized by large nuclei with scant cytoplasm; numerous mitoses are seen in these clusters of cells. The canal buds are not passively surrounded by the rapidly expanding cartilaginous epiphysis; instead, they appear to be actively penetrating into the cartilaginous epiphysis.

The focal invaginations of mesenchymal cells advance toward the central portion of the epiphysis during the tenth through twelfth week of gestation (Figures 2A and 2B). A cluster of mesenchymal cells always appears at the advancing front of the canal. These
cells at the front of the canal abut directly against the cartilaginous matrix and appear to dissolve the matrix between the epiphyseal cartilage cells.

Behind this advancing cluster of mesenchymal cells, the canal progressively forms and becomes more anatomically complex. With the initial formation of the canal, no cellular elements line the canals. The canal exists as an open, acellular channel extending from the perichondrium to the central portion of the cartilaginous epiphysis. No distinct boundary is initially formed between the canal and the cartilaginous matrix.

**Development of the cartilage canals within the epiphysis prior to the initiation of ossification**

By fourteen weeks of gestation, the canals are relatively evenly distributed throughout the epiphysis. With the radial enlargement of the cartilaginous epiphysis, the canals remain "uniformly" distributed throughout the epiphysis. During this period of prenatal development, the canals do not remain static structures, but rather undergo significant changes in size and in the pattern of cellular organization within the canal. Mesenchymal cells away from the advancing front of the canal begin to appear in clusters throughout the canal network. These groups of cells appear within the central portion of the canal, or appear loosely attached to the walls of the canal.

The first change in the cellular organization of the cartilage canal occurs between the twelfth and fourteenth week of gestation. The primitive mesenchymal elements within the canals begin to differentiate into vascular and connective tissue elements and the canals become increasingly complex structures. By twelve weeks of gestation, a portion of the mesenchymal cells form vessels and supporting connective tissue elements. A thin, acellular, hyperchromatic boundary appears in the cartilaginous matrix surrounding the canal (Figure 3). Primitive vascular lumina appear within selective clusters of cells (Figure 4).
Figure 2A. The distal femoral epiphysis after twelve weeks of gestation shows a cartilage canal advancing toward the central portion of the epiphysis. This early canal is only a channel in the cartilaginous anlage; canal is devoid of cellular elements except for the cluster of mesenchymal elements at the terminal end of the canal. (Hematoxylin and eosin, x60)

Figure 2B. Under higher magnification, the cluster of mesenchymal elements in the terminal portion of the cartilage canal are detailed. There are no connective tissue elements or blood vessels contained with the canals at this stage of development. The mesenchymal tissue directly contacts the cartilaginous matrix of the epiphysis (Hematoxylin and eosin, x250)

Over the next two weeks, these mesenchymal elements continue to differentiate into endothelial cells which form distinct arterioles, capillaries, and veins within the anatomic canal. The vascular endothelium may be directly apposed to the cartilage matrix, or it may be separated by a layer of connective tissue elements.

Between sixteen and twenty-two weeks of gestation, the cartilage canals become increasingly complex structures. Mesenchymal elements are not readily evident seen in the canalicular network at this stage of development. Each canal contains at least one
Figure 3. By fourteen weeks of gestation the canals fill with mesenchymal elements which are no longer present only at the terminus of the canal. A hyperchromatic cartilaginous matrix appears at the margins of the canal. (Hematoxylin and eosin, x250).

Figure 4. The mesenchymal clusters of cells begin to arrange themselves in circular patterns and primitive vessel lumen are formed. (Hematoxylin and eosin, x250).

artery and vein separated by a highly organized fibrous tissue stroma (Figure 5). No smooth muscle elements appear along the arterioles. The veins appear as large, dilated structures composed of a single layer of endothelial cells. The fibrous connective tissue elements surround the vascular structures within the canal. The amount of connective
tissue elements within the canal varies considerably; however, no interposing zone of cells or intracellular matrix within the canal separates the tissues of the canal from the chondrocytes and cartilaginous matrix of the epiphysis. The vascular endothelium and loose connective tissues of the canal seem to imperceptibly merge with the surrounding cartilaginous matrix.

The canals have varying diameters throughout the epiphysis. At the periphery of the epiphysis, the vessels within the canals appear larger in diameter and the canals contain more than one arteriole and vein within it. In the more central portions of the epiphysis, the vessels within a canal tend to have a smaller diameter and usually contain only a single arteriole and vein.

Initiation of ossification within the epiphysis

At thirty-two to forty weeks of gestation, initiation of ossification within the central portion of the epiphysis occurs. Changes occur simultaneously within the centrally located chondrocytes and cartilage canals. The central chondrocytes begin to hypertrophy and mitoses are more commonly seen. Significant changes occur simultaneously within the centrally located cartilage canals adjacent to the hypertrophic cartilage cells. The connective tissue elements within the cartilage canals begin to dedifferentiate. The nuclei swell, mitotic divisions are seen and the vessel wall boundaries become less distinct (Figure 6). Mesenchymal tissue elements replace the once highly organized vascular structures (Figure 7).

Once the process of ossification begins in the central region of hypertrophying chondrocytes, the cellular composition of the adjacent cartilage canal continues to change significantly. For the first time, multinucleated giant cells are evident within the cartilage canals (Figure 8). The arterial and venous vascular lumina continue to become less distinct and the canal becomes filled with loosely arranged fibrous tissue elements. The cartilage canals away from this secondary center of ossification remain unchanged throughout this process.
Figure 6. At thirty-two weeks of gestation, changes begin to occur simultaneously in the central chondrocytes cells and in the adjacent centrally located cartilage canals. The chondrocytes begin to hypertrophy. The highly defined vascular and connective tissue elements within the cartilage canals start to dedifferentiate into aggregates of mesenchymal cells. (Hematoxylin and eosin, x150)

Figure 7. At higher magnification, a cartilage canal adjacent to the central region of hypertrophying chondrocytes shows complete breakdown of its vascular structures and clumped mesenchymal tissue elements. (Hematoxylin and eosin, x300)

Development of the secondary center of ossification

As the secondary center of ossification radially expands, larger cartilage canals are incorporated in the ossification center (Figure 9). Well organized cartilage canals remain unchanged at the periphery of the epiphysis and in the cartilaginous portion of the epiphysis between the secondary center of ossification and the growth plate.

DISCUSSION

The relationship between the emerging vascular supply of the cartilaginous anlagen
Once the ossific nucleus of the secondary center of ossification is formed, changes in the cellular composition of the adjacent cartilage canals continue. Multinucleated giant cells and loose connective tissue elements are seen in the canals as well as thin walled vessels. (Hematoxylin and eosin, x150)

The central canals are incorporated into the secondary center of ossification as it expands radially. (Hematoxylin and eosin, x60)

and the development of the primary and secondary centers of ossification occur at different times in prenatal development and through significantly different processes [3, 12, 13, 23]. Several authors [8, 21, 33] have suggested that hypertrophic cartilage cells have
an important role in the development of the vascularization of the epiphysis. Floyd [10] demonstrated that the development of central hypertrophic cartilage cells preceded and was linked to the rapid vascular invasion of the epiphysis in mice. The pattern of canal penetration of the chondroepiphysis, however, is species specific. In contrast to the situation in mice and rats, cartilage canals in humans are present throughout the chondroepiphysis well before the development of the secondary center of ossification [1, 22, 23, 32]. Hurrell [17] proposed that hypertrophic chondrocytes released an angiotrophic agent. Similarly, Kugler [20] hypothesized that hypertrophic chondrocytes induced angiogenesis in connective tissues. Trueta [27, 28, 29] believed that hypertrophic chondrocytes released a vascular stimulating factor that contributed to the initiation of osteogenesis. These authors did not believe that the cartilage canal directly contributed to endochondral ossification.

In the human prenatal distal femoral chondroepiphysis, cartilage canals invade the chondroepiphysis well before any hypertrophic cartilage stimulus. The canals are not passively incorporated into the cartilaginous epiphysis through radial appositional growth of the epiphysis as suggested by Haines [14]. The canals actively penetrate the densely packed chondrocytes of the anlage [7, 9].

Once the canals are formed in the chondroepiphysis, the cellular composition of the canals does not remain static. Rather, the cartilage canals undergo profound changes in their cellular composition and organization prior to the development of the secondary center of ossification. Ganey [11] identified three distinct phases of development of the cartilage canal system in rabbits. We also identified a similar pattern of development in the cartilage canal system in prenatal human specimens.

In the first stage, the cartilage canals are formed by mesenchymal elements extending from the perichondrium and penetrating into the chondroepiphysis. The canals at this stage are acellular channels with mesenchymal cells present only at the terminal portion of the canal [24]. In the second stage of development, the mesenchymal tissue elements increasingly fill the canal and differentiate into a complex network of vascular and connective tissue elements [16, 18]. The third developmental stage begins within the central canals in the chondroepiphysis located adjacent to the hypertrophying chondrocytes [30]. The vascular structures contained within these canals lose their cellular structure and the connective tissue elements dedifferentiate into mesenchymal elements. The canals are then incorporated into the expanding ossifrons.

This study clearly established that the vascular penetration of the distal femoral chondroepiphysis precedes the initiation of ossification in the epiphysis by several months. Hypertrophic cartilage cells have no role in the development of the cartilage canals and the vascularization of the proximal femoral epiphysis in humans. Suppression of vascular ingrowth is not a significant component of the cartilaginous anlage regionally inhibiting the formation of a vascular network.

Cartilage canals provide not only a vascular network to the rapidly expanding chondroepiphysis, but they also supply a source of osteoblastic cell precursors that may contribute to the initiation of the ossification in the chondroepiphysis [6, 11, 17, 19, 25]. The initiation of osteogenesis has been demonstrated to occur adjacent to the central cartilage canals in the distal femoral epiphysis in this study and also in the human talus [1], calcaneus [2] and vertebra [6] and in the canine humerus [31].

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202  

Burkus et al.: Cartilage canals and epiphyseal ossification

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