LOCALIZATION OF IL-17 FAMILY MEMBERS AND COGNATE RECEPTORS IN CHONDROCYTES DURING RAT BONE FORMATION.

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Introduction

The potential for regeneration and repair of musculoskeletal tissues is well known. Fracture healing is a good example of regeneration. During the transient inflammation phase, leukocytes or macrophages are observed. In the repair phase, periosteal cells or mesenchymal stem cells differentiate into chondrocytes or osteoblasts forming woven bone. The newly formed bone is remodeled by osteoclasts. Various growth factors and cytokines play a role in fracture healing. IL-17 is one of the IL-17 family members and is a proinflammatory cytokine expressed by tissues. T-cells expressed IL-17A. The receptors of IL-17 family, including IL-17 receptor (IL-17R) and the newly discovered IL-17 receptor-like molecule (IL-17RL), are also reported to be expressed in chondrocytes. The aim of this study was to examine the hypothesis that IL-17B has an important role in differentiation of chondrocytes.

Materials and Methods

Eight Long Evans rats were used to obtain closed fractures. The mean age of the rats was approximately 12 weeks. A lateral parapatellar knee incision was made to expose the distal femoral condyle and a K-wire was inserted from the troclear groove into the femoral canal. A closed transverse femoral shaft fracture was then created using a three point bending apparatus. At one and two weeks after fracture, the four rats each were euthanized. Fractured femurs were decalcified and embedded in paraffin. Paraffin sections were stained with toluidine blue for histological observation. New born rats were euthanized after 21 days after birth. Proximal ends of tibia were processed for histological study. Sections of fractured femur and growth plate in proximal tibia were also subjected to immunohistochemistry for IL-17A, IL-17B, IL-17R and IL-17RL, using rabbit polyclonal antibodies of IL-17B and IL-17RL, mouse monoclonal antibody of human IL-17A, and Goat polyclonal antibody of human IL-17R. Immunohistochemistry were performed by avidin-biotin alkaline phosphatase complex method. Color was developed with red alkaline phosphatase substrate without any counterstaining. Normal mouse IgG, normal rabbit IgG or TBS were used instead of the primary antibodies as negative controls.

Results

A histological section of fractured femur at one week after fracture showed abundant newly formed callus at the fracture site, including intramembranous ossification and endochondral ossification. Endochondral ossification area is consisted of primitive chondrocytes and prehypertrophic chondrocytes, and sandwiched by newly formed trabecular bone and mesenchymal stromal cells in fibrous tissue. Hypertrophic chondrocytes were not found at this stage, but at two weeks after fracture endochondral ossification area was mostly filled with hypertrophic chondrocytes. IL-17A was localized only in prehypertrophic chondrocytes in the endochondral ossification area, but weakly. On the other hand, the localization of IL-17B was detected strongly in chondrocyte progenitors and prehypertrophic chondrocytes. Osteoblasts in the newly formed trabecular bone and mesenchymal stromal cells at the fracture site were also stained weakly. The receptors of IL-17 were widely localized at the fracture site. IL-17R was localized in primitive and prehypertrophic chondrocytes, osteoblasts in the trabecular bone, and mesenchymal stromal cells in the fibrous tissue. The staining pattern of IL-17RL was similar to that of IL-17R, but the localization of IL-17RL in prehypertrophic chondrocytes was hardly detected. The hypertrophic chondrocytes, which can be seen in the endochondral ossification area at two weeks after fracture, were not stained with any antibodies of IL-17A, IL-17B, IL-17R, and IL-17RL.

Discussion

We examined localization of IL-17 family and the receptors in rat fracture healing and rat proximal tibia including growth plate by immunohistochemistry. In both the fracture site and the growth plate, IL-17A and IL-17B localized in chondrocytes, and IL-17R and IL-17RL also localized in the endochondral ossification area. There was similarity of localization of IL-17 family members and their receptors between fracture healing and bone development in growth plate. The regeneration during fracture healing recapitulates the cartilage and bone morphogenesis in growth and development.

References


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