Chondrocytes from a NOMID Patient Exhibited Resistance to Low Serum Culture Condition and Greater Response to IL1\(\beta\) and TNF Stimulation

Xibin Wang\(^1\), Madji C. Namde\(^1\), Victor Galson\(^1\), Raphaela Goldbach-Mansky\(^2\), Rocky S. Tuan\(^1\)
\(^1\)Cartilage Biology and Orthopaedic Branch, NIAMS, NIH, DHHS, Bethesda, MD; \(^2\)Office of the Clinical Director, NIAMS, NIH, DHHS, Bethesda, MD
tuanr@mail.nih.gov

**Introduction:** Neonatal-Onset Multisystem Inflammatory Disease (NOMID) is a rare autosomal dominant autoinflammatory condition characterized by the triad of urticarial rash, arthropathy, and central nervous system manifestations. The arthropathy is mostly seen in the knee and is present in about half of all patients. The mechanism for the development of the characteristic arthropathy is not clear. In this study, articular and growth plate cartilage biopsies were obtained from one patient undergoing orthopaedic surgery. Chondrocyte cell lines were established from the biopsies. Cell survival was investigated under low serum culture conditions and the gene expression profiles were examined after IL1\(\beta\) and TNF\(\alpha\) stimulation.

**Materials and Methods:** Human tissues were obtained with IRB approval and informed consent of patients. Articular and growth plate chondrocytes were isolated from the biopsies of a NOMID patient. Articular chondrocytes obtained from the intact cartilage of osteoarthritic (OA) patients were used as a control. Chondrocytes were cultured in DMEM/F12 with 1% FBS and cell survival was analyzed with a Live/Death assay. For gene expression analysis, cells were treated for 24 hours with IL1\(\beta\) (5ng/ml) or TNF\(\alpha\) (10ng/ml), total RNAs were isolated, and mRNA levels were quantified by real-time RT-PCR. Gene expression levels were normalized to GAPDH expression. At least three cultures were assayed for each measurement. Numerical data were analyzed using ANOVA followed by Fisher's protected lsd procedure.

**Results:** Results showed that the number of cell death in the control chondrocytes increased after 7 days of culture in a medium with 1% bovine fetal serum, while that of the cells from the NOMID patient remained unchanged (Figure 1.). IL1\(\beta\) and TNF\(\alpha\) treatments increased the expression of IL1\(\beta\), IL-6, NFkB1, MMP-13, and SOX9 in chondrocytes from both the NOMID patient and the control. However, chondrocytes from the NOMID patient exhibited greater increase in the expression of these genes in response to IL1\(\beta\) stimulation. TNF\(\alpha\) treatment also resulted in greater increase in the expression of IL1\(\beta\), MMP-13, and SOX9 in NOMID chondrocytes (Figure 2.).

**Discussion:** Mutations in CIAS1 gene have been found in half of NOMID patients. No mutations were found in CIAS1 gene of the patient in this study. The resistance of NOMID chondrocytes to low serum medium suggests that these cells could exhibit growth advantage in poor nutritional conditions. This characteristic may explain cartilage overgrowth in the arthropathy, given the avascular nature of cartilage.

Sox9 is the master transcription factor for chondrogenesis. Elevation of Sox9 levels leads to increase in cartilage matrix synthesis, which could be another reason of cartilage overgrowth in NOMID patients. MMP13 is an indicator of chondrocyte hypertrophy and precursor of cartilage calcification, which may be associated with heterogeneous cartilage calcification in NOMID patients. Future studies will explore the mechanisms responsible for the alterations in apoptosis and IL1\(\beta\) and TNF\(\alpha\) signaling pathways in NOMID chondrocytes.

**Acknowledgements:** This research was supported by the Intramural Research Program of the NIH, NIAMS (Z01 AR41131).

![Figure 1. Cell death rate of chondrocytes cultured in 1% FBS. (Articular and Growth Plate: chondrocytes from articular cartilage and growth plate of NOMID patient, respectively; Control: articular chondrocytes of OA patient. * indicates p<0.05 in comparison with the means of other groups on the same day).](image1)

![Figure 2. Expression of IL1\(\beta\), IL6, NFkB, MMP13 and Sox9 in articular and growth plate chondrocytes of NOMID patient (Articular and Growth plate, respectively) and in articular chondrocytes of OA patient (Control) in response to IL1\(\beta\) and TNF\(\alpha\) treatments. * indicates p<0.05 in comparison with the mean receiving no treatment (NT)).](image2)