Fibronectin fragments (FN-f) have been identified in synovial fluid and in the damaged cartilage matrix of subjects with osteoarthritis and rheumatoid arthritis (1). FN-f, including the 110kD integrin binding fragment, have been shown to stimulate chondrocyte-mediated cartilage destruction and could play an important role in the progression of arthritis. The effects of FN-f can be either direct, through activation of cell signaling pathways which upregulate MMP expression (2), or indirect through upregulation of cytokines which act on chondrocytes in an autocrine or paracrine fashion. The objective of this study was to use a genomics and proteomics approach to identify potential novel cytokine mediators of cartilage inflammation and destruction induced by FN-f.

METHODS

Human articular chondrocytes, isolated from normal ankle cartilage obtained from tissue donors, were treated with the 110kD FN-f (0.5-1µM) in serum-free monolayer culture as previously described (2). RNA was collected at 6 and 16 hours after stimulation and expression of various cytokine genes was analyzed by cDNA microarray using the Clontech human cytokine array (268 genes). Conditioned media was collected 24 hours after FN-f treatment and used for protein array with the RayBio human cytokine protein array (22 cytokines). It should be noted that this array contained IL-1α but not IL-1β, the latter of which had previously been shown to be upregulated by FN-f (2). Expression of the cytokines identified by the array studies was further examined by RT-PCR in cells stimulated with FN-f and compared to cells stimulated with IL-1α (2ng/ml) and TNFα (10 ng/ml). GAPDH was used as a control and ratios of cytokine/GAPDH were calculated.

RESULTS

Compared to untreated control cultures, stimulation by FN-f resulted in upregulation (>2-fold ratio FN-f/control) of 19/268 (7.1%) of the cytokine and chemokine genes at 6 hours and 22/268 (8.2%) at 16 hours. The gene array results were then compared to results using a cytokine protein array. The most highly expressed cytokines stimulated by FN-f and identified by both gene and protein arrays, were IL-6, IL-8, MCP-1, and GRO-β (Fig.1). A comparison of cytokine and chemokine expression in response to FN-f (0.5µM) for 6 hrs resulted in a 1.5-fold increase in RNA levels for TNFα and IL-1β but not GRO-α, IL-6, IL-8, or MCP-1. The IL-6, IL-8, and MCP-1 genes were upregulated by 0.5µM FN-f (2). Expression of the cytokines IL-6, IL-8, MCP-1, and GRO-β (Fig.1) was further examined by RT-PCR in cells stimulated with FN-f and compared to cells stimulated with IL-1α (2ng/ml) and TNFα (10 ng/ml). GAPDH was used as a control and ratios of cytokine/GAPDH were calculated.

CONCLUSION

The ability of FN-f to stimulate chondrocyte expression of the pro-inflammatory cytokines IL-1, IL-6, IL-8, MCP-1 and GRO family members suggests that damage to the cartilage ECM resulting in fragment formation is capable of inducing a pro-inflammatory state that can be responsible for further progressive matrix destruction. The GRO family proteins are members of the IL-8 superfamily and along with MCP-1 are classified as chemokines due to their known chemotactic activity. IL-8 and GRO proteins are CXC chemokines which attract neutrophils while MCP-1 is a C-C chemokine which attracts macrophages. FN-f stimulated expression of chondrocyte chemokine expression may be particularly important in the pathogenesis of rheumatoid arthritis where pannus formation plays an important role in cartilage destruction. IL-1, IL-6, IL-8, MCP-1, and GRO-α have also been demonstrated in OA cartilage and have catabolic and anti-anabolic effects. The results of this study suggest that targeting the signaling pathways activated by FN-f may be an effective means of inhibiting production of multiple mediators of cartilage destruction.

REFERENCES


CO-AUTHORAFFILIATIONS

**GlaxoSmithKline, King of Prussia, PA
**Arkady Margulis for donor tissues.

ACKNOWLEDGEMENTS

This work was funded by NIH grant AR49003 and a grant from GlaxoSmithKline. Thanks to the Gift of Hope Organ and Tissue Donor Network and Dr. Arkady Margulis for donor tissues.