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Introduction: Surgical samples from herniated disc (HD) show granulation tissue with prominent macrophage and mild T-lymphocyte infiltration concomitant with the onset of neovascularization that is not observed in healthy intervertebral disc. MRI study has shown that epidurally displaced HD tissues more commonly undergo a gradual decrease in the size of HD than those with minimal protrusion. This tendency for natural resorption correlated with the accumulation of Gd-DTPA contrast agent within the vascularized granulation tissues of HD. Changes initiated by contact with infiltrating macrophages may thus contribute to the resorption process. We've then developed a coculture system of disc tissues and macrophages to reproduce the acute phase of disc herniation in vitro. We've previously determined that infiltrated macrophages and disc cells in HD tissues express the matrix metalloproteinases (MMPs) MMP-3 and MMP-7, both of which are potent proteoglycanases. MMP-3 and MMP-7 are strongly upregulated when macrophages are added to disc tissues in our coculture model. We've used this in vitro model system to investigate the roles of MMP-3 and MMP-7 in macrophage infiltration of disc tissue.

Materials and Methods: Murine coccygeal intervertebral discs were isolated from homozygous MMP-3-null (−), MMP-7-null (−) and wild type (wt) 129/Sv mice. Murine macrophages were obtained following intraperitoneal administration of thioglycollate. The isolated murine discs and macrophages were then either cultured separately or in direct contact as co-cultures. Macrophages (1.2X10^6 cells/ml) were suspended in coculture-conditioned media or Opti-MEM and added to the upper wells of chambers. Conditioned media derived from cocultures of macrophages and either wild type or MMP-3-null disc tissue diluted with Opti-MEM was added to the upper and /or lower chambers. After incubation for 5 hours at 37°C, the membrane was removed, washed with PBS, and nonmigrated cells on the upper surface of the membrane were removed. Cells were fixed in methanol for 5 minutes, stained with hematoxylin for 5 minutes, and mounted on glass slides. Cells on the lower surface were counted in 6 high-power fields (X400) per group. Statistical analysis of the data was performed using Mann-Whitney test.

Results: Conditioned medium from discs alone resulted in modest macrophage migration. In contrast, coculture media resulted in a 5.6-fold increase in macrophage migration. When medium was from cocultures of MMP-3-null disc cells and macrophages, migration was reduced to control levels. We conclude that MMP-3 production by disc cells in the presence of macrophages contribute to the generation of an inducing factor of macrophage migration. Macrophage migration by a chondrocyte-secreted factor could involve enhancement of either directional migration (chemotaxis), increased random motility (chemokinesis), or a combination of both. To identify these activities, Boyden chamber assay was performed. Chemokinetic activity, indicated by an increase in migration with increasing concentrations of medium in the upper or the upper and the lower chambers, was observed at high concentrations of conditioned medium from macrophages cocultured with either wild type or MMP-3-null discs. In contrast, chemotactic activity, indicated by increased migration in response to a concentration gradient, was observed only with conditioned medium from macrophage/wild type disc cocultures. MMP-3-dependent factor has chemotactic activity as it fulfills the requirement of directional motility towards a gradient and densensitization of the effect at high factor concentrations.

Discussion: The interesting observation from current study is the role of chondrocyte MMP-3 in the generation of a chemotactic factor that induces macrophage infiltration. MMP-3-null discs did not allow macrophage infiltration in the organ coculture model, and isolated chondrocytes lacking MMP-3 did not allow macrophage infiltration of alginate beads. In addition, coculture medium from wild type macrophages and MMP-3-null discs did not produce macrophage chemoattractants in the checkerboard chemotaxis assay. The nature of the MMP-3-dependent chemoattractant is not clear. Potential candidates include cell surface chemokines such as MCP-1. Chemokine monocyte chemoattractant protein-1 (MCP-1) is a potent macrophage chemoattractant that has been shown to be expressed by disc cells and infiltrated macrophages in human HD samples. Alternatively, MMP-3 mediated degradation of matrix components may release macrophage chemoattractants. We cannot rule out the possibility that MMP-3 inactivates an inhibitory protein that interferes with an endogenous chondrocyte chemoattractant. Irrespective of the molecular nature of the chemoattractant, this is to our knowledge the first example of a definitive role for MMP-3 in a chemotactic process.