INTRODUCTION
HMG-CoA reductase inhibitors (statins) have both lipid lowering and non-lipid lowering pharmacological properties. The latter include a broad range of immunomodulatory and anti-inflammatory effects. These “pleiotropic” effects occur in a number of different cell types including endothelial cells, monocytes, macrophages, vascular smooth muscle cells and T-lymphocytes. For example, statins inhibit matrix metalloproteinase (MMP) expression by activated macrophages that accumulate in atherosclerotic plaques.

The mechanism by which statins exert pleiotropic effects in different cell types appears to be linked to the inhibition of mevalonate synthesis. In addition to reducing cholesterol synthesis, inhibition of HMG-CoA reductase also targets other intermediates of the mevalonate pathway such as the isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (Fig. 1). The reduction of FPP and GGPP and subsequently of the farnesylation and geranylgeranylation of small GTP binding proteins has been directly linked to the mechanism of statin action in non-hepatic cell types. Since one effect of statins is the reduction of MMP expression and secretion from macrophages, we began studies in chondrocytes to address the hypothesis that statins may have beneficial effects for cartilage including the down-regulation of MMP and other catabolic enzyme production.

METHODS
Human OA Chondrocytes Human articular cartilage was obtained from OA donors (Asterand, Detroit, MI). Chondrocytes were isolated via a pronase/collagenase digestion and cultured in alginate beads prior to experimentation. For experimental culture, chondrocytes were plated in high-density monolayer under serum-free conditions. Cells were pretreated with compound +/- IL-1β stimulation. Media was removed following pre-treatment and replaced with media containing fresh compound +/- IL-1β stimulation. Following a 24 hr cytokine stimulation, media was collected, RNA isolated, and gene expression assayed using Taqman RT-PCR. Gene expression results are displayed as relative expression +/- SEM normalized to the expression of Cyclophilin A. MMP-13 protein in culture supernatants was assayed by ELISA (R&D Systems, Minneapolis, MN).

RESULTS
We first explored the effects of statins on collagenase expression in osteoarthritic chondrocytes. In the presence or absence of cytokine stimulation by IL-1β, simvastatin treatment resulted in a concentration-dependent decrease in MMP-13 expression (Fig. 2). The results were similar for another collagenase, MMP-1 (data not shown)

We next explored the mechanism by which simvastatin repressed MMP-13 expression by adding back mevalonate pathway intermediates. Geranylgeranyl pyrophosphate (GGPP), but not farnesyl pyrophosphate (FPP), completely reversed the statin-mediated repression of MMP-13 expression (Fig. 3). The mRNA levels in Fig. 3 were mirrored by MMP-13 protein levels in the culture supernatants (data not shown).

DISCUSSION
Simvastatin reduced the expression of MMP-13 in human OA chondrocytes. The complete reversal of this effect by GGPP suggests that protein geranylgeranylation plays a major role. Partial reversal by FPP suggests that protein farnesylation may also play a role in the complex regulatory networks controlling MMP-13 expression. Significantly, simvastatin and GGTi-2147 also inhibited collagen destruction in IL-1β/OSM-treated human osteoarthritic cartilage explants, a model that recapitulates several key features of OA pathology (data not shown). These data indicate that protein geranylgeranylation regulates collagenase activity and/or expression in human osteoarthritic articular cartilage.