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LETTERS

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The potential negative effect of high-dose glucosamine on the chondrocyte: comment on the article by Caramés et al

To the Editor:

We read with great interest the recent article by Caramés et al, who demonstrated that glucosamine can activate autophagy in human chondrocytes and in mouse knee joints by inhibiting the Akt/FoxO/mammalian target of rapamycin pathway (1). Importantly, the authors proved that intraperitoneal and oral administration of glucosamine induced similar levels of autophagy in the cartilage, thus providing a convenient method for inducing autophagy in vivo in the future. Similarly, another study also demonstrated the inductive effect of glucosamine on autophagy in HeLa and COS-7 cells (2). Glucosamine has been shown to play antiinflammatory, anticatabolic, and antiapoptotic roles in the chondrocyte (3,4), and in the future autophagy might be identified as a mechanism mediating these effects.

However, as mentioned in reports suggesting double roles of autophagy in disease (5), the negative effect of "autophagic cell death" on chondrocytes may need to be elucidated. After treating normal chondrocytes from SD rats with 100 m*M* glucosamine for 24 hours, we observed that the cytoplasm of cells in monolayer culture was filled with numerous vacuoles (Figure 1). When the incubation time was extended, an increasing number of chondrocytes died, which indicated that glucosamine at high concentrations may lead to autophagic cell death. Although we could not determine whether the cells died due to autophagy, the results suggested that high-dose glucosamine may have a negative effect on cell viability.

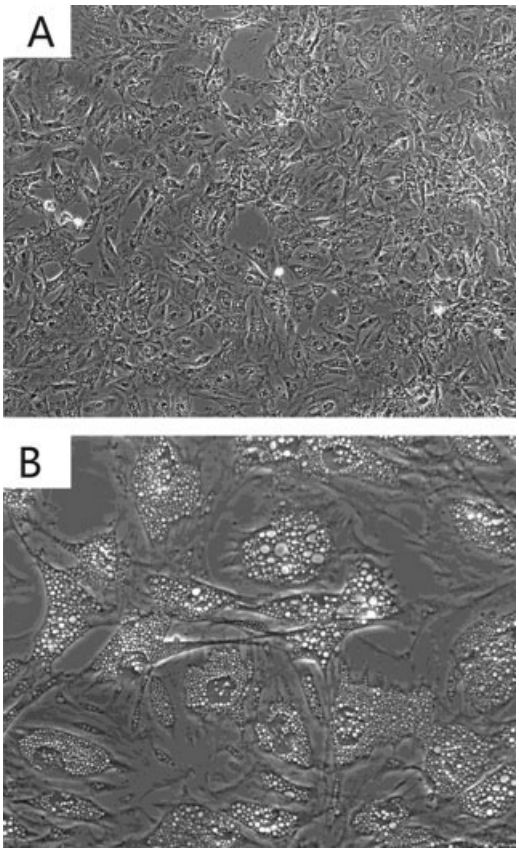

Figure 1. Photomicrographs obtained by phase-contrast microscopy, showing the morphology of chondrocytes treated with 100 m*M* glucosamine for 24 hours, at **A,** lower magnification (100×) and **B,** higher magnification (400×).

In accordance with our observations, De Mattei et al showed that glucosamine at doses of 6.5 mg/ml (~30 m*M*) and 25 mg/ml (~116 m*M*) decreased cell viability in bovine cartilage explants by 28% and >90%, respectively (6). Moreover, ultrastructural analysis showed that the cells in which death was induced by glucosamine exhibited obvious vacuoles in the cytoplasm (6). In addition, Walsh and colleagues reported that after 4 days and 7 days of exposure to glucosamine at a dose of 4.5 mg/ml (~20 m*M*), the DNA content of annulus fibrosus cells was reduced by 75% and 89%, respectively (7). Therefore, it may be imperative to place the emphasis on the concentration when treating chondrocytes with glucosamine.

In summary, although an increasing number of studies have suggested that there may be a role for autophagy in the treatment of arthritis, further studies, especially in the human system, are still needed to rule out any negative effects.

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Protein kinase Cδ mutations may contribute to lupus through effects on T cells: comment on the article by Belot et al

To the Editor:

Belot et al recently described 3 siblings with juvenile-onset systemic lupus erythematosus, all of whom had a missense mutation in *PRKCD*, encoding protein kinase δ (PKCδ) (1), similar to previous observations in PKCδ-deficient mice in which lupus developed (2). B cells from these siblings were

resistant to B cell receptor– and Ca^{2+}-induced apoptosis and demonstrated increased proliferation, also similar to B cells in PKCδ-deficient mice. The development of autoimmunity is interpreted as demonstrating that PKCδ is crucial in regulating B cell tolerance and preventing self-reactivity in humans. We would like to note that PKCδ activity is reduced in patients with sporadic non-Mendelian lupus, and that this inactivation in T cells can contribute to the development of lupus-like autoimmunity through effects on T cell DNA methylation (3).

DNA methylation is an epigenetic mechanism that silences gene expression by promoting a repressive chromatin configuration that is inaccessible to transcription factors. DNA methylation patterns are established during differentiation and serve to suppress genes that are irrelevant or detrimental to the functions of mature cells. The methylation patterns are then replicated each time a cell divides, by the maintenance methyltransferase DNA methyltransferase 1 (DNMT-1). DNMT-1 levels are low in resting T cells but are up-regulated in stimulated T cells by signals transmitted through the ERK pathway. Inhibiting DNA methylation in dividing T cells, either with direct DNMT inhibitors such as 5-azacytidine or the lupus-inducing drug procainamide, or with MEK inhibitors such as PD98059 and U0126, alters gene expression and converts normal antigen-specific CD4+ T cells into autoreactive, cytotoxic, and proinflammatory T cells that are sufficient to cause lupus-like autoimmunity in animal models (4,5). The role of decreased T cell ERK pathway signaling in causing lupus-like autoimmunity has been confirmed in a double-transgenic mouse model in which dominant-negative MEK can be induced selectively in T cells by adding doxycycline to the drinking water. In these mice, doxycycline decreases T cell DNMT-1 levels, demethylates DNA, activates genes normally suppressed by DNA methylation, and causes lupus-like autoimmunity (6–8).

PKCδ is a multifunctional signaling molecule that plays a crucial role in ERK pathway signaling in T cells (9). Inhibiting T cell PKCδ with rottlerin or the lupus-inducing drug hydralazine, or by transfection with a dominant-negative PKCδ similarly demethylates T cell DNA methylation and alters gene expression (3). CD4+ T cells treated with hydralazine are sufficient to cause lupus-like autoimmunity in mice (10). Taken together, these findings indicate that inhibiting ERK pathway signaling, through effects on PKCδ or downstream signaling molecules, is sufficient to cause a lupus-like disease.

Importantly, PKCδ is catalytically inactivated by oxidative damage in CD4+ T cells from patients with active lupus, causing decreased ERK pathway signaling, decreased DNMT-1 levels, and demethylation of the same genes normally silenced by DNA methylation (11). These observations suggest that PKCδ mutations may contribute to lupus through effects on T cells, and the effects on B cells seen in the patients described by Belot and colleagues as well as in PKCδ-deficient mice may augment these effects.

Dr. Richardson serves on the scientific advisory board of Ignyta.

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Reply

To the Editor:

We thank Dr. Richardson and his colleagues for their comments. In our study, we demonstrated that a missense *PRKCD* mutation (c.1528G>A; p.G510S) leads to resistance in B cell receptor– and calcium-dependent apoptosis, resulting in increased B cell proliferation. Our findings are consistent with the recent demonstration that PKCδ is involved in an ERK-dependent signaling pathway of B cell negative selection through Ca^{2+}-dependent apoptosis (1). In addition, adoptive transfer and transgenic B cell receptor (BCR) experiments in *PRKCD*-knockout mice showed B cell proliferation and BCR-dependent autoimmunity (2,3). Kuehn et al described a patient with *PRKCD* mutations and demonstrated that the knockdown of *PRKCD* in lymphocytes was associated with increased proliferation of B cells but not T cells, thus seemingly emphasizing the major role of B cells in disease pathogenesis (4). Our experiments did not show evidence of either a deficiency of T cell ontogenesis or an activation anomaly of T cells. In contrast, B cells and natural killer cells do demonstrate impaired differentiation (Belot A: personal observations).

DNA demethylation is an interesting mechanism that has been widely studied in autoimmune diseases. This process is not restricted to CD4 T cells, having been implicated in B cells or epithelial cells in the context of systemic lupus erythematosus (SLE) and Sjögren's syndrome (5,6). In accordance with the work performed by Richardson and colleagues in this field, we can speculate that *PRKCD* mutations may impact on DNMT-1 and CD4 T cell demethylation. Thus, further "methylome-related" experiments are warranted to determine whether T cell dysfunction is relevant to the pathogenesis of this new Mendelian cause of SLE. At this time, however, we would hold that the case remains unproven.