



Undenatured Type II Collagen Mechanism of Action

	Page
Executive Summary	2
Animal Research	3
In Vitro Research	4

Executive Summary

UC-II® undenatured type II collagen is a patented form of collagen with undenatured (native) type II collagen for joint health support. A small amount (40 mg/day) is believed to work by inducing a process known as oral tolerance that ultimately engages the immune system in the repair of its own joint cartilage.

Oral tolerance is an immune process that allows the body to distinguish between innocuous compounds such as dietary proteins and intestinal bacteria and potentially harmful foreign invaders. In the digestive tract, oral tolerance occurs in the gut-associated

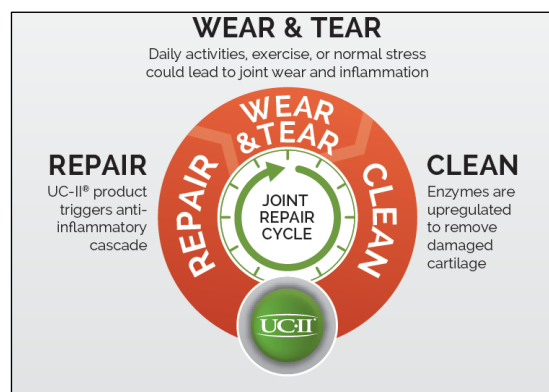
lymphoid tissue (GALT), considered to be the body's most abundant lymphoid tissue. The GALT is primarily composed of mesenteric lymph nodes and patches of lymphoid tissue surrounding the small intestine called Peyer's patches. It is in the Peyer's patches, which contain an organized collection of immune cells, where most of the immune responses in the digestive tract are generated. Through a cascade of immunological events, Peyer's patches take in and screen compounds from the gut lumen and, depending on the compound, turn the body's immune response on or off (Weiner et al¹).

Researchers believe UC-II® undenatured type II collagen induces a form of oral tolerance that results from exposure to a compound in small amounts. More specifically, **UC-II®** undenatured type II collagen is believed to be transported across the gut epithelial cells to the underlying immune cells in the Peyer's patches where it activates naive T cells to become T regulatory (Treg) cells that specifically target type II collagen. The activated Treg cells then migrate from the GALT through the lymphatic system and enter circulation. When they recognize a compound similar to what was ingested (e.g., type II collagen in joint cartilage), the Treg cells secrete anti-inflammatory cytokines such as TGF-beta, interleukin (IL)-4 and IL-10. This action suppresses the action of cells involved in the normal breakdown of collagen and other extracellular matrix proteins (Gupta et al²).

Animal studies support oral tolerance as the mechanism of action of UC-II® undenatured type II collagen and demonstrate that the UC-II® undenatured form of type II collagen is effective. In animal models of rheumatoid arthritis (Tong et al³ and Nagler-Anderson et al⁴), small amounts of oral undenatured type II collagen have been shown to increase the production of key cells and cytokines associated with the induction of oral tolerance while reducing arthritis symptoms. Of particular note is the reported increase in activated Treg cells and cytokines favoring anti-inflammatory pathways. Moreover, small, but not large, amounts of undenatured collagen showed efficacy.

In vitro studies further support oral tolerance as the means by which UC-II® undenatured type II collagen may exert joint health benefits. In one study (Asnagli et al⁵), researchers characterized the activated Treg cells specific for type II collagen, revealing the production of anti-inflammatory cytokines, including a high level of IL-10. In another study (Müller et al⁶), IL-10 was found to play a key role in reducing the destructive effects of TNF-alpha, an inflammatory cytokine elevated in osteoarthritis that impairs chondrocyte survival and cartilage function. Separately, researchers have confirmed that **UC-II®** undenatured type II collagen contains the biologically active epitopes required to induce oral tolerance (Bagchi et al⁷), and that these epitopes are resistant to the gastric acids and digestive enzymes found in the stomach.

Together, these findings suggest that **UC-II®** undenatured type II collagen may exert its joint health benefits via oral tolerance thereby initiating anti-inflammatory and cartilage protective pathways that prevent the immune system from damaging its own joint cartilage.



Animal Research

Undenatured type II collagen protects against joint damage in an animal model of rheumatoid arthritis in a manner consistent with oral tolerance, while the denatured form has no effect.

In this animal study,⁴ Nagler-Anderson and colleagues compared the effect of pre-feeding DBA/1 Lac J mice with either undenatured or denatured bovine type II collagen on symptoms of collagen-induced arthritis (CIA). CIA is an experimental autoimmune disease that can be elicited in susceptible strains of mice and other animals by immunization with type II collagen, the major constituent protein of joint cartilage. Following immunization, the animals develop an autoimmune polyarthritis that shares several clinical and histological features with rheumatoid arthritis (RA) and is thus considered an ideal animal model for RA.

For this study, the researchers elicited CIA-induced arthritis in the mice by immunization via skin injection of type II collagen in Freund's complete adjuvant. For the oral treatments, undenatured collagen was prepared by the technique of Trentham et al⁸ and denatured collagen was prepared by high heat processing.

Results indicate that the oral intake of undenatured type II collagen, prior to immunization, significantly ($P < .05$) suppressed the incidence of CIA after Day 30, compared to control. By contrast, the oral intake of denatured type II collagen had no observable effect on the incidence or severity of the disease.

These findings suggest that the oral intake of *undenatured* type II collagen may be required to exert joint health benefits. Moreover, the efficacy of short-term feeding of undenatured type II collagen in small amounts is consistent with the induction of the bystander suppression form of oral tolerance.

Undenatured type II collagen provides symptom relief in an animal model of rheumatoid arthritis, an action attributed to inducing oral tolerance and modulating inflammatory pathways.

In this animal study,³ Tong and colleagues investigated the effect of the oral intake of chicken type II collagen on T cells from mesenteric lymph node (MLN) lymphocytes in rats with collagen-induced arthritis (CIA). CIA was induced in male Sprague-Dawley rats immunized with type II collagen in Freund's complete adjuvant. The researchers fed the rats type II collagen (10, 20, and 40 mcg/kg/day for 7 days) from Day 14 to 21 after immunization. Arthritis was evaluated by hind paw swelling and a polyarthritis index, and MLNs and synovium were harvested for histological examination. Key cells (e.g., Treg and Th17) and cytokines (e.g., IL-2, IL-4, IL-17, and TGF-beta) that are involved in either the induction of disease or the induction of oral tolerance were measured.

At all dosages given, type II collagen suppressed secondary inflammatory reactions and histological changes in the rats. More specifically, it significantly decreased the pro-inflammatory mediators (IL-2, IL-17) while increasing anti-inflammatory mediators (IL-4, TGF-beta) produced by the MLN lymphocytes from the rats, and significantly increased the proportion of Treg cells relative to the proportion of Th17 cells, an indication of anti-inflammatory effects.

These findings suggest that the oral intake of type II collagen helps relieve arthritis symptoms by inducing oral tolerance and modulating inflammatory pathways.

In Vitro Research

T regulator cells specific for type II collagen are characterized by secretion of anti-inflammatory cytokines.

In this in vitro study,⁵ Asnagli and colleagues investigated the secretion profile of cytokines from T regulator (Treg) cells specific for type II collagen. Using Treg clones, isolated and expanded from collagen-specific TCR transgenic mice, the researchers found Treg cells are able to mediate contact-independent immune suppression and were characterized by a specific cytokine profile.

More specifically, the Treg cells secreted a high level of interleukin 10 (IL-10), an anti-inflammatory cytokine, and an intermediate level of interferon-gamma (INF-gamma), a cytokine involved in Treg cell migration. The Treg clones also had a high expression of several markers of cell activation in a manner similar to natural Treg cells.

These findings indicate that Treg cells specific for type II collagen have a specific cytokine profile, including a high level of anti-inflammatory IL-10, which plays a key role in the cells' ability to induce oral tolerance.

In human chondrocytes, the anti-inflammatory action of IL-10 protects against damage from TNF-alpha, a pro-inflammatory cytokine elevated in osteoarthritis.

In this in vitro study,⁶ Müller and colleagues investigated the ability of IL-10 (an anti-inflammatory cytokine) to offset the catabolic effects of TNF-alpha (a pro-inflammatory cytokine) in human articular chondrocytes.

In osteoarthritic cartilage, TNF-alpha is elevated, which suppresses the synthesis of cartilage-specific extracellular matrix required for the survival of chondrocytes and the normal function of cartilage. However, IL-10 is also elevated, suggesting a possible role in counteracting the catabolic effect of TNF-alpha.

For this study, primary human articular chondrocytes were treated with either recombinant IL-10, TNF-alpha or a combination of both. Alternatively, these primary cells were transduced to overexpress human IL-10 and then treated with TNF-alpha for 6 or 24 hours. The effect of IL-10 was then assessed based on the expression of cartilage-specific matrix proteins (e.g., collagen type II, aggrecan, matrix-metalloproteinases [MMP]-3, and MMP-13) and pro-inflammatory cytokines.

Results indicate that transduced chondrocytes significantly up-regulated their collagen type II expression. The subsequent TNF-alpha stimulation suppressed type II collagen and aggrecans production, but increased MMP and cytokine expression in the chondrocytes compared to the non-stimulated controls.

These findings suggest that IL-10 modulates some of the catabolic effects of TNF-alpha in chondrocytes and plays a role in the homeostasis of cartilage-specific extracellular matrix needed for chondrocyte survival and cartilage function.

UC-II® contains biologically active epitopes with the ability to induce oral tolerance.

Using a validated enzyme-linked immunosorbent assay (ELISA),⁷ Bagchi and colleagues have demonstrated that **UC-II®** undenatured type II collagen contains glycosylated biologically active epitopes with the correct composition and structural conformity of galactose-dependent glycoprotein. Further, electron microscopy analysis revealed the epitopes are a triple helix structure that is preserved in gastric juice. More specifically, after incubation in simulated gastric juice for 90 min, virtually 100% of the **UC-II®** undenatured type II collagen active epitope was recovered as either soluble protein or insoluble matrix.

The researchers propose **UC-II®**

undenatured type II collagen works via these epitopes as they survive digestion with the three-dimensional structure needed to interact with the lymphoid tissue surrounding the small intestine (e.g., Peyer's patches). In this way,

UC-II® undenatured type II collagen is believed to affect the gut immune system, inducing oral tolerance to turn off the immune response targeting type II collagen in joint cartilage.

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