Effect of Pre-Loading Oral Glucosamine HCl/Chondroitin Sulfate/Manganese Ascorbate Combination on Experimental Arthritis in Rats

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The therapeutic effect of a nutritional supplement consisting of a combination of glucosamine hydrochloride (FCHG49), purified sodium chondroitin sulfate (TRH122), and manganese ascorbate (GCM)[3] was investigated in the rat model of collagen-induced autoimmune arthritis (CIA). The GCM compound was mixed with a palatable nutritional paste (Nutri-cal® [NC]). Oral administration of the NC/GCM compound was initiated in 26 rats 10 days before immunization and continued until the day of sacrifice. One group of 12 control rats was given no oral agents; a second group of 12 control rats received NC only. Evaluations included arthritis index (AI) scoring by three independent evaluators, histologic index (HI) scoring of lesions, T-cell proliferation, and serological studies for antibody classes and subclasses. Both the AI and HI criteria showed a statistically significant reduction in the prevalence of CIA in rats pretreated with the NC/GCM (54%) compared to the combined control groups (96%, χ^2 analysis $P = 0.001$). Rats fed the NC/GCM also exhibited a significant decrease in the severity of autoimmune arthritis in both the AI and HI compared to control Group 2 (immunized-NC) (χ^2 analysis $P < 0.05$). Histological studies verified the decreased incidence of arthritis in the NC/GCM group compared to control Group 2.

GCM treatment failed to alter T-cell proliferation and antibody production to bovine type-II collagen, indicating that its effects are not due to alteration of the antigen-specific immune response. [E.B.M. 2001, Vol 226:144–151]

Key words: glucosamine hydrochloride; sodium chondroitin sulfate; arthritis

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[3] GCM is available as Cosamin®, a patented product of Nutramax Laboratories, Inc., Edgewood, MD. FCHG49 and TRH122 are registered trademarks of Nutramax Laboratories®, Inc., for the proprietary specifications for glucosamine HCl and chondroitin sulfate, respectively.

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Rheumatoid arthritis (RA) is a significant health problem in terms of numbers of sufferers (U.S. incidence 23.7/100,000) and in degree of associated disability as well (1). The disease is characterized by a chronic and destructive inflammatory reaction, possibly autoimmune in etiology, attacking the synovial membranes of joints and many other tissues of the body. Much has been learned in recent years about the pathogenesis of RA. Genetic predisposition and exogenous arthritogens are believed to contribute to a T-cell-mediated autoimmune reaction to one or more constituents of the joint. Some researchers believe that the inflammatory process in RA is an inappropriate response to an infectious agent, which could theoretically share cross-reactive epitopes with an endogenous molecule such as type-II collagen (2).

In normal articular cartilage, chondroitin sulfate is produced by chondrocytes and synoviocytes from the precursor amino sugar, glucosamine. Once excreted into the connective tissue matrix, these compounds spontaneously assemble into proteoglycan aggregates. The electrochemical properties of these proteoglycan aggregates and their relationships with collagen type-II in the matrix are responsible for the properties characteristic of connective tissues, such as resiliency and viscosity (3, 4). Because of their vital role in the structure and function of cartilage, it should not be surprising that abnormalities in the matrix are a significant contributor to the pathology of RA (2, 5, 6). In the past decade, matrix-modifying agents, including the endogenous molecules, glucosamine and chondroitin sulfate, have been the subjects of increased research (7–9). A growing body of evidence documents a stimulatory effect of glucosamine supplementation on chondrocytes and synoviocytes (10, 11). Similarly, dietary supplementation of chondroitin sulfate has been shown to decrease degradation of matrix components *in vitro* and to decrease inflammation *in vivo* (12, 13).

In addition, manganese is a necessary cofactor for the

in vivo biosynthesis of matrix components including chondroitin sulfate and for mitochondrial superoxide dismutase, which inhibits oxidative damage in tissues (14, 15). Furthermore, manganese is an essential trace element that has a role in mucopolysaccharide synthesis and in activating glycosyltransferase enzymes that attach modified sugars to proteins (collagen) and each other (14, 16). Ascorbate is combined with the manganese to support the cross-linkage among collagen matrix fibers, which are damaged in cases of ascorbate deficiency, thus reducing the tensile strength of the assembled fibers.

We therefore undertook a study of the possible beneficial effect of a compound containing glucosamine HCl, chondroitin sulfate, and manganese ascorbate (GCM) in an experimental model of RA. The compound used in our study has been evaluated in cell culture work and has been shown to elevate levels of circulating agents that stimulate cartilage metabolism and inhibit degradation (17). The combination also was shown to be safe and clinically effective as well as in animals (18–23) and clinically effective in humans with osteoarthritis (OA) (24, 25). RA and OA share pathological characteristics that include varying degrees of inflammation and degeneration (26–29). Therefore, it is not unreasonable to expect agents that are structure modifying may be of benefit in both diseases.

This model of collagen-induced arthritis (CIA) is recognized as an *in vivo* tool in researching the mechanisms of RA. In CIA, first described by Trentham *et al.* (30), injections of native type-II collagen produce arthritis in 40–100% of immunized laboratory rodents (30, 31). Dark agouti (D/A) rats are especially susceptible to the disease, and they develop a chronic and severe form of autoimmune arthritis, which leads to permanent bone destruction and ankylosis of affected joints. The chronic, progressive destruction is believed to be the consequence of both cell-mediated and humoral immune responses, which mimic the pathogenesis of RA in humans (32).

The goal of this study was to determine the effect of a dietary supplement, consisting of glucosamine hydrochloride, sodium chondroitin sulfate, and manganese ascorbate, on the incidence and severity of type-II collagen-induced autoimmune arthritis in D/A rats.

Materials and Methods

Animals and Study Groups. Fifty-two female D/A ($RT1^{av1}$) rats from Harlan Laboratories (Indianapolis, IN), 8–9 weeks of age, body weight range of 154–173 g, were sedated with Metophane®, ear-tagged, and divided randomly into three experimental groups. Group 1 (control-immunized-no oral agents) comprised 12 rats that were immunized with bovine type-II collagen. Group 2 (control-immunized-Nutri-cal® [NC]) consisted of 12 rats that received only Nutri-cal® (NC) 2 ml orally every 12 hr in addition to being immunized. Group 3 (treatment-immunized-NC/GCM) had 26 rats that received CGM homogenized with NC 2 ml orally every 12 hr and were also immunized. Two normal rats (not-immunized-no-oral agents) were reserved for histologic and antibody comparison with the immunized rats (total rats = 52). Rats in Groups 2 and 3 were pre-loaded for 10 days with the oral agents prior to immunization. All rats were injected with 300 μg of bovine type-II collagen (Sigma Chemical Company, St. Louis, MO), emulsified in complete Freund's adjuvant (Sigma) intradermally in three separate locations at the tail head on Day 0. Intraperitoneal booster immunizations were administered on Day 7 using 300 μg of bovine type-II collagen in phosphate buffered saline (PBS). On the 42nd day, all rats were sedated, weighed, bled, and sacrificed, and tissues were collected for histologic examination.

Test Materials. The studied compound (GCM) was in the form of a white powder containing 200 mg of sodium chondroitin sulfate (TRH122), molecular weight = 16,900 (derived from bovine tracheal cartilage), 250 mg of glucosamine HCl (FCHG49) (derived from chitin of shellfish origin), 5 mg of manganese, and 33 mg of ascorbate in a gelatin capsule (Cosamin®, Nutramax Laboratories, Inc., Baltimore, MD). The powder was removed from the capsules, homogenized using a power mixer, and combined with a commercial veterinary nutritional supplement paste (Nutri-cal®, Evsco Laboratories) (NC). NC is a highly palatable oral supplement used in veterinary medicine to provide either partial or full nutritional support for mammals. This feeding technique avoided the inherent stress associated with restraint and gastric tubing. Mixing the GCM with NC allowed *accurate* dosing of the agents with each administration. We believe that the accurate dosing improved the study design and data evaluation. Because no dosage studies for GCM are available, we elected to administer the highest concentration of GCM the rats would readily consume. Rats in the NC only group received 2 ml orally every 12 hr. Rats in the NC/GCM group received the 2 ml of NC homogenized with a dosage of 1.4–1.6 g of glucosamine HCl/kg (250 mg of glucosamine HCl/rat), 1.15–1.3 g of low molecular weight sodium chondroitin sulfate/kg (200 mg of sodium chondroitin sulfate/rat), 0.029–0.032 g of manganese/kg (5mg/rat), and 0.18–0.21 g of ascorbate kg (33 mg/rat) orally every 12 hr. These amounts were equivalent to 1 capsule of Cosamin® regular strength per rat every 12 hr.

Blood Collection. Five hundred microliters of whole blood via tail bleed was allowed to clot at 4°C; sera were separated via centrifugation at 10,000 rpm (Beckman Tabletop Centrifuge) and stored at −70°C. The blood samples were collected on Days 0, 7, 14, 21, 28, 35, and 42.

Antibody Assessment. The antibody data were collected to determine if GCM affected the humoral immune response. Serum samples were thawed immediately prior to use and antibody to type-II collagen and its class (IgA, IgM, IgG) or subclass (IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}) was determined by enzyme-linked immunosorbent assay (ELISA) methodology using a multipoint analysis procedure.

The IgG antibody response was computed by adjusting

the optical density (OD) readings using a standard adjustment formula to compensate for interplate variation. The adjustment formula sets up a relationship between the positive and negative controls run on each plate and the OD value for each sample. The use of the same positive and negative control sera for every plate allows the adjustment and comparison of OD values from different plates. This formula used follows:

$$100/(\text{Positive control} - \text{Negative control}) = X/\text{Sample OD reading}.$$

The IgG isotype response was recorded based upon the optical density reading. The average optical density was calculated for each group.

Animal Observation and Body Weight. Rats were observed every 12 hr to check their health status. Evidence of arthritis, location of lesions, and a description of the lesions were documented, as were abnormal feedings. Rat body weights were determined upon receipt and at weekly intervals on Days 7 through 42.

Arthritis Index (AI). An arthritic index (AI) scoring system was used as described in the *Handbook of Animal Models of the Rheumatic Diseases* (33). Each rat had a maximum possible score of 22 points. Inflammation of a carpal joint and/or digit was scored 1 point each. Inflammation of the tarsal joints was scored up to 2 points each, depending on the severity of the inflammation. Scoring of arthritis was done on Days 35 and 42 post-immunization by three independent and blinded evaluators by examining the rats for evidence of arthritis in all limbs and joints. A joint was determined to be inflamed if there was swelling, redness, and/or pain as manifested by lameness. The mean score of the three evaluators was used for all statistical analysis.

Histologic Index (HI). The HI was developed by the co-author (S.L.H.) a board-certified veterinary pathologist. At the time of sacrifice, tissues were collected from each rat and placed into separate vials containing 10% neutral buffered formalin. The tissue sections were coded to prevent bias and scored blindly by the co-author (S.L.H.). The following joints and tissues were evaluated: forelegs-carpal, carpometacarpal metacarpal phalangeal and phalanges, and hindlegs-stifle, tarsal, tarsometatarsal, and phalanges. Grades for carpometacarpal, tarsometatarsal, metatarsal phalangeal, and phalangeal joints were based on the highest lesion grade observed for the multiple digits examined. The total maximum score possible/rat for all joints evaluated = 72/rat.

Histological grades for each of the 18 joints were are as follows:

1 = minor focal infiltrate (<20%);
2 = moderate infiltrate (20%–30%);
3 = severe infiltrate (30%–50%), no pannus or erosion of cartilage;
4 = very severe infiltrate (>50%), pannus and/or cartilage erosion and fibrosis.

Data Analysis. Summary statistics were used to describe the physical appearance, body weight changes, arthritis and histologic index. Box and whisker plots were constructed to display the distribution of the arthritic index and histologic index by group. The difference in the incidence of arthritis between the combined control and treatment groups was assessed using the χ^2 test. Differences in the AI and HI between the 3 groups were tested using the Kruskal–Wallis one-way analysis of variance by ranks (non-parametric). Pairwise differences were tested using a multiple comparisons procedure with overall significance level of 0.05 (34).

The correlation between the arthritis index and histologic index was calculated using the Spearman rank-correlation coefficients to provide additional support for the relationship between the two methods of evaluation.

RESULTS

Gross and Histological Evaluation. The total time of the experiment: 52 days, including 10 days of preloading oral agents prior to immunization. Forty-nine of the original 52 rats in the study survived through the time of sacrifice. Three rats died during the study, one from Group 1 and two rats from Group 3. Those rats were excluded from further analysis. Post-mortem examination determined the cause of death to be anesthetic-related. No adverse events were attributable to the feeding of either the vehicle (NC) (Group 2) or the treatment (NC/GCM) mixture (Group 3). When the arthritis progressed, the affected rats became increasingly inactive and withdrawn, tending to remain at the back of the cage. Rats that developed arthritis showed reduction in body weight. The overall reduction in weight by Day 42 was as follows: Group 1, 5% ± 4%; Group 2, 9% ± 5%; Group 3, 7% ± 6%, respectively.

The first signs of arthritis appeared on Day 19 post-immunization (PI) and continued until Day 42 (Fig. 1). The clinical assessment of joint swelling and redness (arthritis index, AI) revealed that the rats fed NC/GCM (Group 3) showed a delay in the development of arthritis compared to the untreated control group (Group 1). The prevalence of joint swelling varied somewhat between the control groups since rats fed NC also showed a delay in the development of arthritis compared to animals receiving no treatment. Rats fed NC/GCM (Group 3) exhibited a lower prevalence of arthritis based on AI evaluation, compared to animals receiving no supplement (Group 1) by Day 21, but there was no difference between Group 2 and Group 3 concerning the prevalence of arthritis until Day 30.

By Day 28, the prevalence of arthritis was similar in the two control groups (1 and 2), whereas the percent of rats with arthritis in the NC/GCM group remained lower. This trend continued and by Day 42 the overall percentage of rats developing arthritis in the two control groups combined was statistically higher than in the (GCM) group (22/23 [96%] and 13/24 [54%], respectively [$P = 0.001$] χ^2).

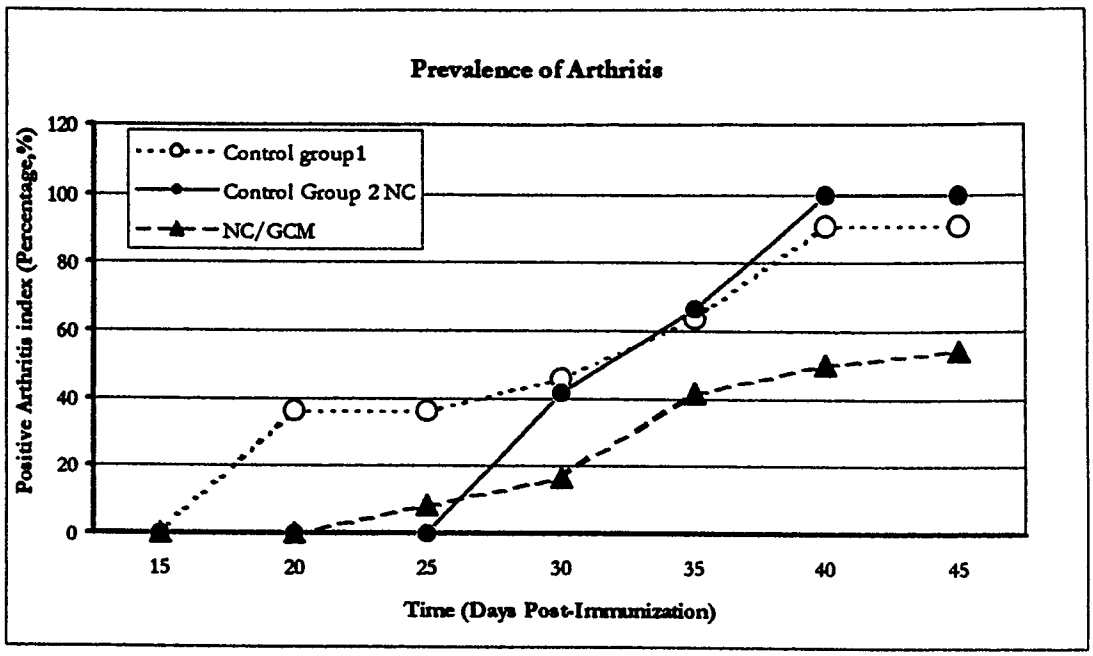

Figure 1. Prevalence of arthritis. Female D/A rats immunized with bovine type-II collagen were divided into three groups: positive control animals that did not receive any oral agents (Group 1, $n = 12$) (○); rats treated with NC only (Group 2, $n = 12$) (●); and rats orally dosed with NC/GCM (Group 3, $n = 26$) (▲). Animals were observed on a twice daily basis for the development of arthritis. The percentage of rats with lesions is recorded. NC/GCM treatment significantly decreased the prevalence of joint disease compared to the positive control and NC-treated rats beginning on Day 30 ($P = 0.001$, χ^2 analysis).

Analysis of the AI data by the Kruskal–Wallis one-way analysis of variance by ranks indicated statistically significant differences between the three groups (Day 35, $\chi^2 = 9.25$ with 2 df [$P = 0.009$]; Day 42, $\chi^2 = 11.00$ with 2 df [$P = 0.004$]). Multiple comparisons testing of pairwise differences revealed significant differences between Groups 2 and 3 only. The clinical assessment of joint disease revealed that the severity of arthritis, as determined by AI scores, was statistically different between the NC (control Group 2) and the NC/GCM (Group 3) on Days 35 and 42 (median scores, 5.5 versus 0.0 and 13.1 versus 3.5, respectively [$P < 0.05$]) (Fig. 2a,b). Thus, at the later time points, animals fed the dietary supplement (CGM) showed a reduction in the severity of joint swelling and redness (AI) compared to the rats fed the vehicle only (NC).

Analysis of the histologic index (HI) data using the Kruskal–Wallis one-way analysis of variance by ranks indicated statistically significant differences between the three groups (Fig. 3). Multiple comparisons testing analysis further showed no difference between control Group 1 and Group 3 (NC/GCM). However, the rats in the NC/GCM treatment group did have a significantly lower median severity of HI compared to the rats in the vehicle only (NC) group (median scores 12.0 versus 28.5, respectively [$P < 0.05$]).

A strong positive correlation was observed between the AI and HI scores ($r_s = 0.82$, $P < 0.001$). Animals that were negative for clinical disease (negative AI) were also found to have negative histological evidence. Figure 4a is a photomicrograph of a section of normal rat stifle showing the junction of the joint capsule, distal femur, and proximal tibia with the associated articular cartilage. For comparison, Fig. 4b represents a photomicrograph from the same region of a rat with CIA and shows a grade 4 lesion based on the histologic index criteria as previously outlined. The inflamed arthritic joint had an abundance of proteinaceous fluid in the joint space. The synovial membrane, usually lined by one cell layer, is intensely cellular and mimics lymphoid tissue containing vessels, macrophages, clumps of plasma cells, and aggregates of lymphocytes forming lymphoid follicles.

Immunologic Evaluation. *Antibody assessment.* Anti-bovine type-II collagen antibodies serve as an indicator of the development of an autoimmune response (29). In this study the serum was evaluated for antibody response to bovine type-II collagen using ELISA (data not shown). We examined the IgG, IgM, and IgA responses separately. Similar collagen-specific IgA, IgM, and IgG responses among the three experimental groups were observed. The IgG antibodies against type-II bovine collagen were detectable beginning on Day 14 post-immunization in all three groups, but GCM administration delayed production of IgG antibody, so that the group showed lower levels of IgG bovine type-II collagen antibody compared to the combined control rats.

Evaluation of the average bovine type-II collagen-specific IgG isotype levels in the treatment group revealed a decrease in IgG1 antibody levels beginning on Day 14, extending to Day 28 in the NC and NC/GCM groups compared to the untreated controls. On Day 42, the NC/GCM

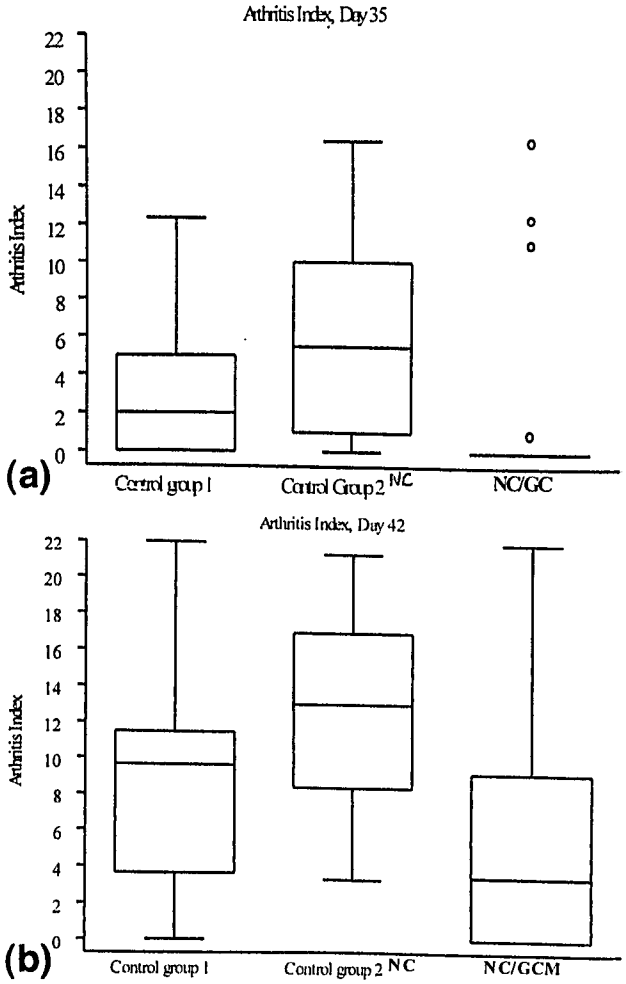

Figure 2. Arthritis index, Days 35 and 42. The weekly arthritis index (AI) was calculated for each individual rat by assessing the presence and severity of joint inflammation affecting the digits, carpal and tarsal joints. Box plots of AI indicate the interquartile range, median range, and outliers for the positive control (Group 1), NC (Group 2), and NC/GCM (Group 3). On Day 35 (a) and Day 42 (b), the NC/GCM group showed significantly lower arthritis index compared to the NC group ($P < 0.05$). However, the NC/GCM and control Group 1 results were not significantly different ($P > 0.05$).

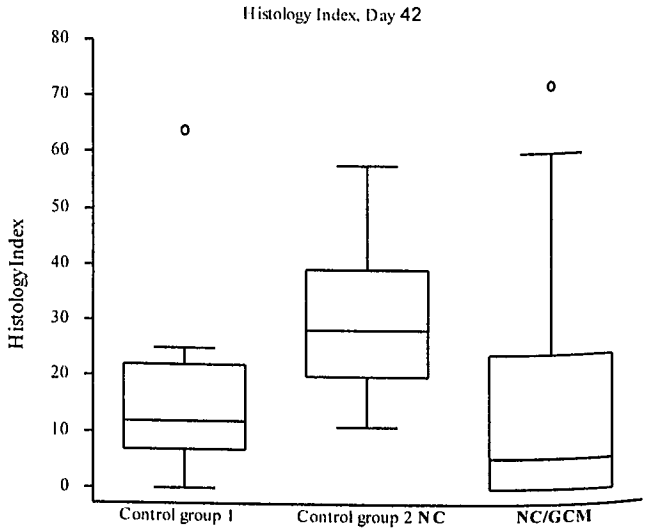

Figure 3. Histopathologic index. The histopathologic index (HI) was calculated for each rat by scoring the severity of inflammatory infiltrates and articular cartilage destruction in joints of the fore and hind limbs. The severity was based on a scale of 0–4 with Grade 0 representing no lesions and Grade 4 representing an intense inflammatory infiltrate with accompanying pannus and underlying articular cartilage destruction. The HI is the sum of the joint lesions from four limbs with a maximal possible score of 72 per rat. Box plots of HI indicate the interquartile range, median, range, and outliers for the control Group 1, NC (Group 2), and NC/GCM (Group 3) at sacrifice (Day 42). The NC/GCM-treated rats did show a significant decrease in the median severity of joint lesions compared to control Group 2 (NC) ($P < 0.05$). However, the NC/GCM and control Group 1 results were not significantly different ($P > 0.05$).

rats continued to show a decrease in IgG1 bovine type-II collagen antibody levels compared to the untreated control rats. On Day 28 both the NC and NC/GCM groups showed greater levels of bovine type-II collagen-specific IgG_{2b} antibody compared to the positive control animals, possibly accounting for the lack of difference noted in the total IgG antibody levels. All other IgG isotypes tested (IgG_{2a} and IgG_{2c}) failed to demonstrate a difference between the treatment groups.

T-cell proliferation. No differences were found on Day 42 between the groups in T-cell proliferation of spleen or lymph node cells to type-II bovine collagen (data not shown).

DISCUSSION

The purpose of this study was to examine the possible therapeutic effect of GCM on a model of rheumatoid arthritis in D/A rats immunized with bovine type-II collagen. We hypothesized that the purported joint matrix modifying ability of the GCM in osteoarthritis would reduce the development and severity of autoimmune arthritis. The effect of this compound was evaluated using clinical (AI) and histopathological (HI) criteria for the assessment of joint involvement. Rats fed NC/GCM had a lower incidence (n = 13/24: 54%) of joint lesions based on both the clinical (AI) and histopathologic (HI) evaluation compared to control animals (n = 22/23: 96%) (P = 0.001). However, we did not find a reduction in the severity of lesions based on either the AI or HI criteria between the control Group 1 (immunized-no oral agents) and the NC/GCM rats (Group 3). Control Group 2 receiving the NC alone had a higher median HI (28.5) than control Group 1 (12.0). The increased severity of pathology in the NC group versus control Group 1, although not statistically significant ($P > 0.05$), may be due to the higher level of nutrition resulting in a greater immune response to the type-II collagen immunization. If the NC actually resulted in an exacerbation of the lesions, then the rats receiving the combination of NC/GCM (Group 3) may have had less severe arthritis if the NC had not been used as a means of administering the GCM. Thus, it is the difference between the NC group and the NC/GCM group that demonstrates a statistically significant therapeutic effect of GCM. Both the AI and HI evaluation revealed a significant reduction in the severity of arthritis between the NC control group and the NC/GCM group ($P < 0.05$).

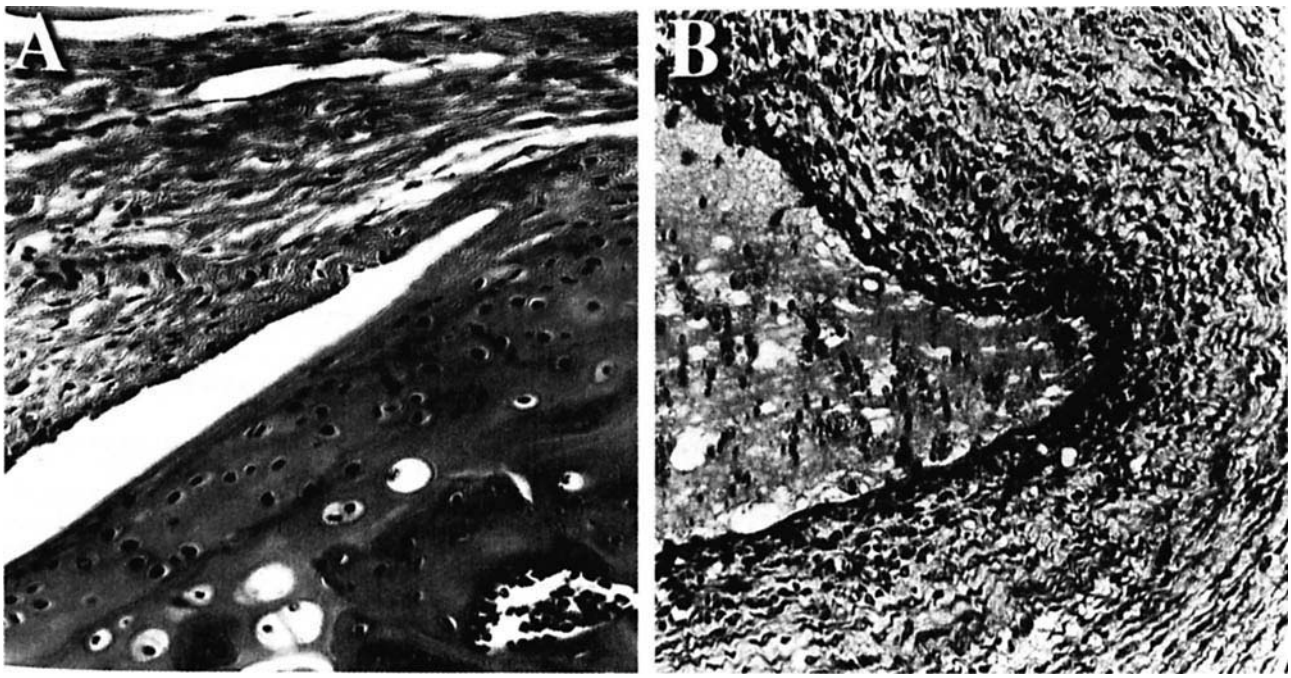

Figure 4. Knee joint. (a) Hematoxylin-and-eosin-stained 5-micron longitudinal section through the knee joint of a normal rat and (b) similar section in a rat exhibiting arthritis. The arthritic joint has increased proteinaceous fluid and abundant neutrophils in the joint space. The synovial lining is thickened with an increase in type-II synovial epithelium and adjacent infiltrates of lymphocytes and plasma cells (original magnification 100x).

We examined and graded the arthritis cases in this study using both the Arthritis Index (AI) and Histologic Index (HI) scoring system. The use of both techniques ensures a thorough evaluation of lesions and decreases the likelihood of occult lesions. Correlating the two systems of scoring arthritic lesions increases confidence in the experimental results. There was a strong positive correlation between the AI and HI ($r_s = 0.82$, $P < 0.001$). These data held a consistent relationship across the three experimental groups in both the prevalence (HI) and severity of lesions (AI and HI).

The observed effects may be explained in several ways, including immune modulation and joint matrix modification. We evaluated the effect of GCM on immune-mediated damage by examining circulating levels of bovine type-II collagen antibody and by assessing antigen-specific T-cell proliferation.

After immunization, all rats, regardless of treatment, had IgG antibodies against type-II bovine collagen. Although there was a slight delay in the production of antibody in the animals given NC/GCM, this difference may indicate that the antibodies are a reflection of tissue injury. There was a difference between the groups in the antibody response indicated by the reduction in total IgG and IgG_1 levels in rats treated with NC/ (Group 2) and GCM (Group 3) (data not shown). The results suggest that nutritional status at the time of immunization played a major role in controlling bovine type-II collagen antibody production. There appeared to be a correlation between bovine type-II collagen-specific IgG_1 antibody levels and the severity of joint lesions on Day 42 ($r_s = 0.696$). We also tested T-cell proliferation to the antigen on Day 42 and found no differences among the three groups.

Oral administration of either chondroitin sulfate or glucosamine has been shown to be beneficial in the treatment of arthritis (35–39). Chondroitin sulfate affects lysosomal enzyme activities as well as hyaluronan and glycosaminoglycan synthesis and possibly degradation. In the examination of joint fluid from patients with osteoarthritis it was concluded that chondroitin sulfate failed to prevent the influx of leukocytes into the joint cavity although the polysaccharide has been shown to exhibit anti-inflammatory properties (35).

Investigators have also examined the anti-arthritic and anti-inflammatory properties of glucosamine sulfate compared to indomethacin using rat models of mechanical and adjuvant-induced arthritis. Glucosamine sulfate was found to be as effective in abrogating joint inflammation. Setnikar *et al.* in a separate study more closely examined the anti-inflammatory properties of glucosamine sulfate using footpad injections of clearly defined pro-inflammatory agents (i.e., serotonin, bradykinin, and histamine) (38). Glucosamine was not effective in preventing inflammation and biochemical changes in the joint fluid induced by these substances. The agent, however, decreased the generation of superoxide radical by peritoneal macrophages *in vitro* and inhibited liver lysosomal enzymes *in vivo*.

We suggest that the observed response is mediated through the direct effect of the compound on the joints. Chondroitin sulfate has documented anti-inflammatory effects, apparently through its inhibition of metalloproteinases such as collagenase and proteoglycanase (13). A recent

study (22) has shown a significant anti-inflammatory effect of the studied agents in an acute synovitis model in dogs when the animals were pretreated before the induction of the synovitis. The stimulatory effect of glucosamine on synoviocytes, which has been verified in cell culture (10, 11), would contribute to maintaining the structure and function of the synovial lining. Another study has shown that although glucosamine and chondroitin sulfate operate by different mechanisms they are synergistic in stimulating chondrocyte proteoglycan synthesis (40). The same study also showed based on an instability animal model, that the combination retards cartilage degeneration more effectively than glucosamine or chondroitin sulfate alone. The combined result of these actions was a decrease in both the incidence and severity of arthritis. These reports and our findings provide some support for the hypothesis that the combination of glucosamine HCl/chondroitin sulfate/manganese ascorbate reduces immune mediated arthritis by reducing the susceptibility of the joint to matrix immune attack.

The findings in this report are encouraging and support the need for further studies. Extending the experimental duration to 90 days would examine the long-term efficacy of GCM. Additional experimental modifications, such as administering of the study agents after induction of arthritis, would be indicated since patients usually present with chronic advanced inflammatory joint disease. Our results suggest that GCM treatment is beneficial in the treatment of immune-mediated joint disease, probably through its joint modifying and anti-inflammatory properties.

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