

Angiogenesis Inhibition Suppresses Collagen Arthritis

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Summary

Neovascularization is observed in a spectrum of diseases such as solid tumors, diabetic retinopathy, and rheumatoid arthritis. It is also evident in rat collagen-induced arthritis (CIA), an animal model with histologic, clinical, and radiographic manifestations resembling rheumatoid arthritis. To evaluate the effects of angioinhibition in CIA, Louvain rats were immunized with type II collagen to induce arthritis and then administered an angiogenesis inhibitor, AGM-1470, in an attempt to either prevent arthritis or suppress established disease. Using clinical and radiographic criteria, AGM-1470 prevented CIA and significantly suppressed established disease without evidence of immunosuppression. Histologic sections from control ankle joints manifested pannus and neovascularization, which were absent in experimental animals. This is the first study to investigate this novel agent in an autoimmune disease, and additional evaluation of this promising compound in other diseases that are potentially angiogenesis dependent, such as rheumatoid arthritis, might be warranted.

Neovascularization plays a physiologic role in wound healing and corpus luteum development (1). Pathologic neovascularization is also critical for solid tumor growth, proliferative diabetic retinopathy, and pannus formation in rheumatoid arthritis (2). Inhibitors of neovascularization, or "angiogenesis inhibitors," have been evaluated in vitro and in tumor models in vivo, although the effect of these novel agents on inflammatory arthritis has not been previously described (3). In this study, the effect of AGM-1470, an angiogenesis inhibitor, on collagen-induced arthritis (CIA) was examined. CIA is a T cell-dependent animal model of rheumatoid arthritis in which rodents (4) and nonhuman primates (5) develop a polyarthritis after immunization with type II collagen (CII). In rats, arthritis typically appears 10–14 d post-CII immunization. Pathologically, CIA resembles rheumatoid arthritis with pannus formation, cartilage/bone erosions, and new blood vessel formation. Humoral and cellular immune responses to CII are present in both diseases (6). AGM-1470 is a synthetic derivative of fumagillin, a naturally occurring fungal antibiotic of *Aspergillus fumigatus fresenius*. In the current study, the ability of AGM-1470 to prevent or diminish CIA was determined by clinical, radiographic, and histologic methods.

Materials and Methods

Experimental Design. Female Louvain (LOU) rats (125–150 gm) were used in all experimental protocols. In an attempt to induce arthritis, rats were immunized intradermally using ether anesthesia on day 0 with 0.5 mg chick CII (Genzyme, Boston, MA) solubi-

lized in 0.1 M acetic acid and emulsified in IFA (Difco Laboratories, Detroit, MI) (4). Synovitis typically develops 10–14 d postimmunization in 90–100% of control rats. AGM-1470 was solubilized in 10% ethanol and 5% gum arabic/normal saline mixture. 0.3 ml of AGM-1470 was given subcutaneously on alternate days using a dose of 27 mg/kg. Four treatment protocols (A–D) were used with control rats receiving no active compound. In protocols A–C, AGM-1470 was initiated on day 2, before the onset of arthritis, in an attempt to prevent CIA. AGM-1470 was discontinued on day 26 (protocol A), 16 (protocol B), and 8 (protocol C). In protocol D, AGM-1470 was instituted on day 10 (arthritis onset) and treatment continued to day 26 to suppress existing arthritis.

Arthritis Assessments. Both the incidence and severity of the arthritis were evaluated. Incidence was defined as the number of rats that had clinical evidence of joint inflammation during the study period, and severity was quantified by scoring daily each paw in integers from 0 to 4 (0 = normal, 4 = maximum) based on increasing levels of swelling and periarticular erythema. The sum of the scores for all four paws was calculated as an arthritic index (4), with a maximum possible score of 16 per rat. Since CIA primarily affects hind limbs, scores of 6–8 represent severe arthritis.

Radiographic scoring was assessed by the extent of joint space narrowing, bone destruction, periosteal new bone formation, and soft tissue swelling (7). Scores were assigned as integers from 0 to 3 per limb (0 = normal, 3 = maximum joint destruction) and were determined by a blinded investigator. The radiographic joint index represents the sum of hind paw scores from each rat (8), with a maximum possible score of six per rat. Ankle joints were harvested at the completion of the protocols on day 28, decalcified, and stained with hematoxylin and eosin.

Cellular and Humoral Immunity. Delayed-type hypersensitivity (DTH) to native CII was quantified in vivo by a radiometric ear assay (8). Radiometric ear indices >1.4, which represents >2 SD

above the mean for naive controls, are considered significant DTH responses to CII. IgG antibodies to CII were measured in selected serum samples obtained on day 28 by an ELISA (9).

Statistics. Continuous variables were analyzed by their group means (student's *t* test) and dichotomous variables by their proportionate group frequencies (χ^2 test). The Yates correction factor was used where appropriate. Results were considered significant at $p < 0.05$.

Results

In an attempt to prevent CIA, AGM-1470 was injected subcutaneously using three injection protocols beginning before the onset of CIA (protocols A-C). None of the rats in protocols A and B developed arthritis, and only one of nine rats administered AGM-1470 in protocol C had arthritis (delayed in onset to day 17) compared with 28 of 30 with arthritis in the group control ($p < 0.00000001$, prevention protocols A + B + C vs. controls) (Table 1). Rats that were treated between days 2 and 8 (protocol C) were evaluated for an additional 2 mo after completion of the standard 28-d AGM-1470 protocol. No subsequent onset of CIA was noted during this prolonged period of further observation. To evaluate the effect of AGM-1470 on established arthritis, the compound was given on alternate days from the day of arthritis onset until the end of the study (protocol D). All of these rats developed arthritis by day 10 and were begun on AGM-1470 treatment at that point. Within 8 d (day 17), there was a significant reduction ($p < 0.002$) in arthritis severity, which was maintained throughout the remainder of the study period (Fig. 1).

Blinded radiographic scores of the hind limbs were significantly lower in AGM-1470 treated rats compared with controls ($p < 0.001$) (Table 1). Histologic evaluation of hind limbs obtained from control rats revealed pannus formation with neovascularization throughout the inflammatory infiltrate (Fig. 2 A). Neovascularization and pannus were absent in hind limb sections obtained from AGM-1470-treated rats (Fig. 2 B). The levels of sensitization to CII, as measured by DTH and IgG antibody levels on day 28, were high in both control and experimental animals (Table 1). Aside from the single

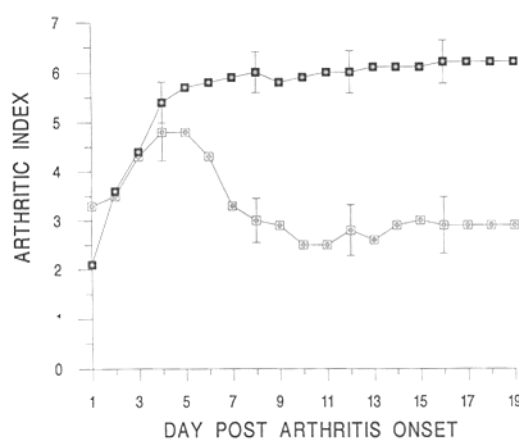


Figure 1. Arthritis severity in rats with established arthritis treated with AGM-1470. (■) Controls; (□) AGM-1470-treated rats. There was a significant diminution in arthritis severity within 8 d after initiation of AGM-1470 therapy in experimental animals (group D), compared with arthritic controls (Table 1, $n = 28$) ($p < 0.002$), which was sustained throughout the remaining study period.

arthritic rat, all experimental animals appeared healthy throughout the study with normal weight gain and with normal scar formation at the immunization sites. In addition, there were no toxic side effects such as hair loss or infection, commonly associated with agents that might have immunosuppressive or antiproliferative activity.

Discussion

AGM-1470 is the first angiogenesis inhibitor to be evaluated in an autoimmune disease. Using clinical, radiographic, and histologic criteria, it effectively prevented and suppressed CIA. In previous *in vivo* studies of tumor growth, the compound has been shown to diminish neovascularization. In contrast, AGM-1470 had no significant effect on ascitic (angioindependent) leukemic cell growth, suggesting that it is not simply an antiproliferative agent. In the current study, the lack of neovascularization in the synovium and the excellent

Table 1. Arthritis Incidence, Radiographic Scores, and Immune Responses in AGM-1470-treated and Control Rats

Protocol	Arthritis incidence	Radiographic index*	Antibody to CII [‡]	DTH to CII [§]
Controls	28/30 (93%)	4.6	0.20	3.37
A + B	0/10 (0%)	0	0.17	3.75
C	1/9 (11%)	ND	ND	ND
D	10/10 (100%) [¶]	1.5	ND	ND

* Mean radiographic scores at the conclusion of the study, day 28.

‡ Mean OD at 490 nm of a 1:2,560 dilution.

§ Mean radiometric ear index.

|| $p < 0.001$ compared with controls.

¶ All rats manifested arthritis before institution of AGM-1470 treatment.

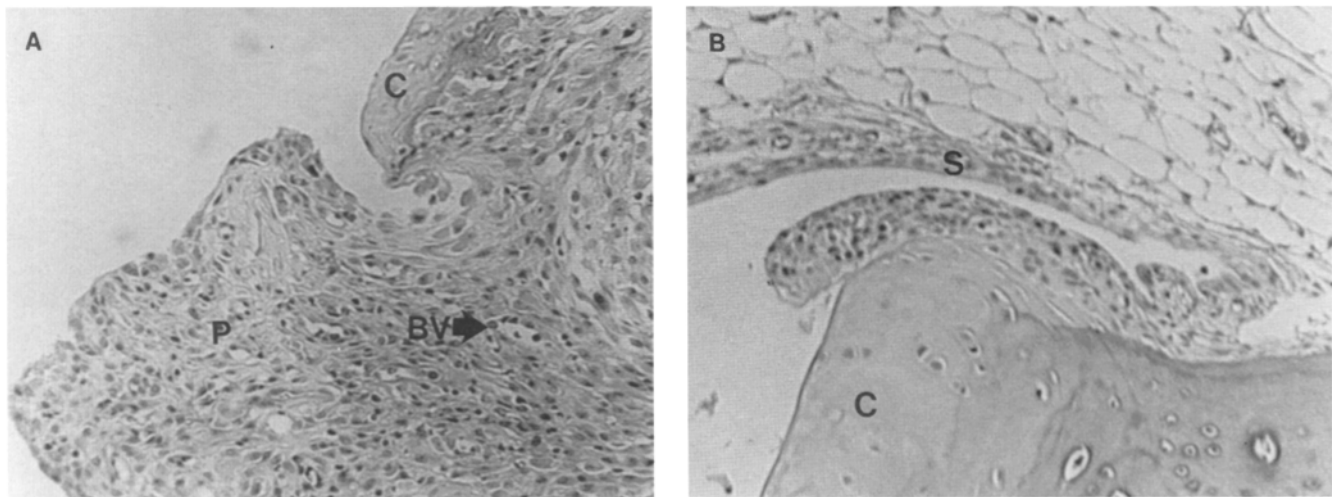


Figure 2. Photomicrographs of ankle joints from control (A) and group A AGM-1470 (B) treated rats. (A) Note the proliferative synovium (pannus), inflammatory cells, and blood vessels, which are not evident in AGM-1470-treated rats (B). BV, blood vessel; C, cartilage; P, pannus; S, synovium. $\times 400$.

immune responses to CII in experimental rats indicate that AGM-1470 also functions as an angioinhibitor in CIA and not as an immunosuppressant. Angiogenesis appears to be critical for both the induction and maintenance of CIA. When AGM-1470 was given for a brief interval between days 2 and 8 (protocol C), it prevented the development of synovitis for at least 2 mo. Given this long time frame, it is unlikely that the compound was slowly released from tissue stores. A more plausible mechanism is that angioinhibition in the first 10 d of CIA precluded pannus formation. This concept is supported by the observation that blood vessels are first observed within the CIA synovium 5–7 d after CII immunization (10). Although complete reversal of existing arthritis did not occur, clinical severity was significantly diminished by AGM-1470, possibly because endothelial cell turnover is rapid in neovascularization compared with static vessels. Angioinhibition may affect endothelial cells directly or via indirect stimulation/inhibition of various cytokines such as thrombospondin, TNF- α , IFN- α_2 , fibroblast growth factor, TGF- α , and

TGF- β (11–15). Alternatively, angioinhibitors may suppress proteinases, such as TIMP-1, which are necessary for basement membrane penetration by budding endothelial cell capillary loops (16).

Disease-modifying antirheumatic drugs have been used to treat severe rheumatoid arthritis and a few have been reported to have minor angioinhibitory capacities in vivo (17, 18), although the significance of this is unclear since most have immunosuppressive properties as well. Unfortunately, these agents are only partially successful in the treatment of rheumatoid arthritis and have major side effects. Because of the efficacy of AGM-1470 in CIA, evaluation of this novel angioinhibitor in rheumatoid arthritis might be warranted. It could also be synergistic in combination therapies utilizing immunosuppressive and antiinflammatory agents. AGM-1470 and similar compounds may, therefore, offer an entirely new treatment option for a spectrum of angiogenesis-dependent rheumatic diseases.

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