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Inflammation and Collagenase Production in Rats with Adjuvant Arthritis Reduced with 13-*cis*-Retinoic Acid

Abstract. *Oral administration of 13-*cis*-retinoic acid (40 or 160 milligrams per kilogram of body weight daily) significantly reduced the inflammation associated with developing and established adjuvant arthritis, an experimentally induced arthritis in rats that resembles human rheumatoid arthritis. The amount of collagenase secreted in tissue culture by adherent cells isolated from the inflamed joints of adjuvant rats treated with 13-*cis*-retinoic acid also decreased as compared to the amount secreted by cells from vehicle-treated adjuvant rats. Collagenase is important in the joint destruction accompanying rheumatoid arthritis. The successful use of retinoids in the treatment of this proliferative but nonmalignant disorder demonstrates a new application of these compounds.*

In recent years, naturally occurring all-*trans*-retinoic acid and the synthetic retinoid 13-*cis*-retinoic acid have been used to treat a variety of disorders. All-*trans*- and 13-*cis*-retinoic acid have been effective in the prevention of experimental neoplasms (*1*); 13-*cis*-retinoic acid, the less toxic compound, has been used in the management of human tumors (*2*) and in the treatment of a number of proliferative dermatologic disorders (*3*). Rheumatoid arthritis is another proliferative but nonmalignant disease in which cartilage, tendons, and subchondral bone are progressively destroyed by a mass of proliferating and invading synovial cells. Collagenase and prostaglandin E_2 (PGE_2) are synthesized and secreted in large quantities by rheumatoid synovial cells, and these two compounds are prime mediators of the joint destruction seen in rheumatoid disease (*4*). Until recently, only corticosteroids were known to block the production of collagenase by these cells.

Brinckerhoff *et al.* have reported that all-*trans*- and 13-*cis*-retinoic acid also inhibited the production of collagenase by monolayer cultures of rheumatoid synovial cells; PGE_2 production was affected little or not at all (*5*). We report here the effect of oral treatment with 13-*cis*-retinoic acid on the inflammation associated with adjuvant arthritis (AA) in rats and on the production of collagenase and PGE_2 by adherent cells taken from inflamed ankle joints of these rats (*6*). Adjuvant arthritis, which can be induced by the injection of adjuvant into one hind paw of a rat, shares a number of features with rheumatoid arthritis, for example, proliferating synovitis, joint swelling, and erosion of cartilage and bone (*7*). The condition is characterized by an initial inflammatory response that peaks in the adjuvant-injected paw by day 5; a systemic response that is characterized in part by the development of chronic inflammatory lesions in both the injected and the contralateral hind paw develops at about day 14. The secondary stage of AA is thought to result from a cell-mediated immune response either to disseminated *Mycobacterium* antigens or, more likely, to some endogenous antigen (*8*). The concentrations of acute-phase proteins, for example, fibrinogen (*9*), in plasma from rats with AA are elevated as they are in plasma from patients with rheumatoid arthritis (*10*). Adjuvant arthritis is widely used as a model in which to identify and to test the efficacy of anti-inflammatory compounds. Nonsteroidal anti-inflammatory drugs and corticosteroids markedly inhibit the development of AA when administered from the time of adjuvant injection or when given to animals with established AA (*11*).

The data illustrated in Fig. 1 (combined from three experiments) demonstrate that treatment with 13-*cis*-retinoic acid (40 or 160 mg/kg) for 25 days suppressed the development of AA. Treatment with 13-*cis*-retinoic acid suppressed the secondary lesions that developed in the arthritic paws (both adjuvant-injected and contralateral paws) between days 11 and 25 (Fig. 1, A and B) to a greater extent than it did the acute lesion that developed in the injected paw during the first days of the disease (Fig. 1A).

The concentration of plasma fibrinogen in normal rats was 298 ± 16 mg per deciliter of plasma. In vehicle-treated rats with AA, this rose to 586 ± 48 mg/dl but decreased significantly to 373 ± 34 mg/dl in rats with AA treated with 13-*cis*-retinoic acid at a dosage of 160 mg/kg. The rats with AA gained significantly less weight than normal rats during the 25-day experimental period, and this effect was not reversed by treatment with 13-*cis*-retinoic acid.

13-*cis*-Retinoic acid was also effective in the treatment of established AA. The AA was allowed to develop for 21 days, and then rats with equal mean paw volumes were divided into three groups for treatment. Drugs administered once a day by intubation for 8 days were either vehicle, indomethacin (3 mg/kg), or 13-*cis*-retinoic acid (100 mg/kg). Changes in paw volume and in the concentration of

Table 1. Effect of treatment for 8 days with 13-*cis*-retinoic acid or indomethacin on established adjuvant arthritis. Means ± the standard errors of the means are reported for the combined data from two experiments. In these experiments, arthritis was induced by the subcutaneous injection of adjuvant into the base of the tail. Changes in paw volume (the volume on day 28 minus the volume on day 21) are the combined values for both hind paws. Drugs were administered from day 21 through day 28.

Treatment (*N*)	Dose (mg/kg)	Change in paw volume (ml)	Plasma fibrinogen* (mg/dl)
Vehicle (11)		+1.14 ± 0.25	1482 ± 92
13-*cis*-Retinoic acid (11)	100	−0.25 ± 0.11†	965 ± 97†
Indomethacin (11)	3	−1.92 ± 0.28†	369 ± 61†

*Measured as described by Exner *et al.* (*22*). †Significantly different from the value for vehicle-treated arthritic animals ($P < 0.05$).

plasma fibrinogen were measured to assess drug effects on the established AA (Table 1). Both drugs suppressed the AA. At the doses administered, indomethacin was considerably more effective than 13-*cis*-retinoic acid. The doses of 13-*cis*-retinoic acid required to suppress developing and established AA were quite high; however, as far as we know, no data are available concerning the bioavailability of 13-*cis*-retinoic acid under the conditions of these experiments. Recent experiments suggest that 13-*cis*-retinoic acid may be more potent as an anti-inflammatory agent in AA when administered in an oily vehicle (for example, peanut oil) instead of in aqueous vehicle as in these experiments (data not shown).

Adherent cells isolated from the inflamed synovia of the rats in one of the 25-day experiments were tested for their ability to synthesize collagenase and PGE_2 in vitro (Table 2). Synovia from arthritic rats that had been treated with either vehicle or 13-*cis*-retinoic acid for 25 days were excised, dissociated enzymatically (*12*), and cultured as monolayers for 48 hours. We measured the concentrations of collagenase and PGE_2 in the media by using radiolabeled fibrils of reconstituted collagen as substrate for collagenase (*13*) and a radioimmunoassay for PGE_2 (*14*). Cells from normal rats secreted comparatively low concentrations of both collagenase and PGE_2. The cells from rats with AA treated with retinoic acid secreted less collagenase than cells from vehicle-treated rats with AA. The PGE_2 concentrations in these cultures were similar for all arthritic groups. These data are consistent with earlier studies concerning the effects of 13-*cis*-retinoic acid on collagenase and PGE_2 production by primary cultures of rheumatoid synovial cells (*5*). In those studies a 4- to 6-day exposure of synovial cells in culture to all-*trans*- or 13-*cis*-retinoic acid ($10^{-6}M$ or $10^{-7}M$) lowered the concentration of collagenase in the culture medium to undetectable levels, while the concentration of PGE_2 decreased only slightly. In the experiments described here, 13-*cis*-retinoic acid was not added to the cultures in vitro. Therefore, the finding that suppression of collagenase synthesis was only modest may reflect a loss of retinoid from synovial tissues during the dissociation process and, subsequently, a low drug concentration in the cultures. The inhibition by retinoic acid of collagenase production by synovial cells in culture is a reversible phenomenon (*15*).

The mechanism by which 13-*cis*-retinoic acid acted to decrease the inflammation and production of collagenase associated with AA is not clear. Retinoids may act directly on the polymorphonuclear leukocytes found in rheumatoid synovium (*4*) to suppress inflammation (*16*). The possible involvement of PGE_2 deserves consideration, since, in addition to being an important mediator of certain aspects of the inflammatory response, for example, edema, hyperemia, and pain, PGE_2 may also modulate cell-mediated immunity (*17*). Retinoids have been shown to both increase and decrease PGE_2 synthesis, depending on the type of cells studied, the retinoid, and its concentration (*18*). In our experiments,

Table 2. Effect of oral administration of 13-*cis*-retinoic acid on collagenase and PGE_2 production by synovial cells taken from rats with adjuvant arthritis. On day 25, monolayers of synovial cells were established from groups of rats and were cultured in triplicate in Dulbecco's modified Eagle's medium with 10 percent fetal calf serum for 48 hours (*12*). Harvested medium was assayed for collagenase and PGE_2 (*13, 14*). Values represent means ± the standard errors of the means.

Rat group (*N*)	Treatment	Collagenase (μg collagen degraded per hour per mg cell protein)	PGE_2 (ng per mg cell protein)
Normal (3)		< 1	10 ± 2
Arthritic (6)	Vehicle	243 ± 30	4900 ± 45
Arthritic (6)	13-*cis*-Retinoic acid (40 mg/kg)	139 ± 34*	4750 ± 1539
Arthritic (6)	13-*cis*-Retinoic acid (160 mg/kg)	174 ± 34†	5700 ± 793

*Compared to cells from vehicle-treated rats ($P < 0.02$). †Compared to cells from vehicle-treated rats ($P < 0.10$).

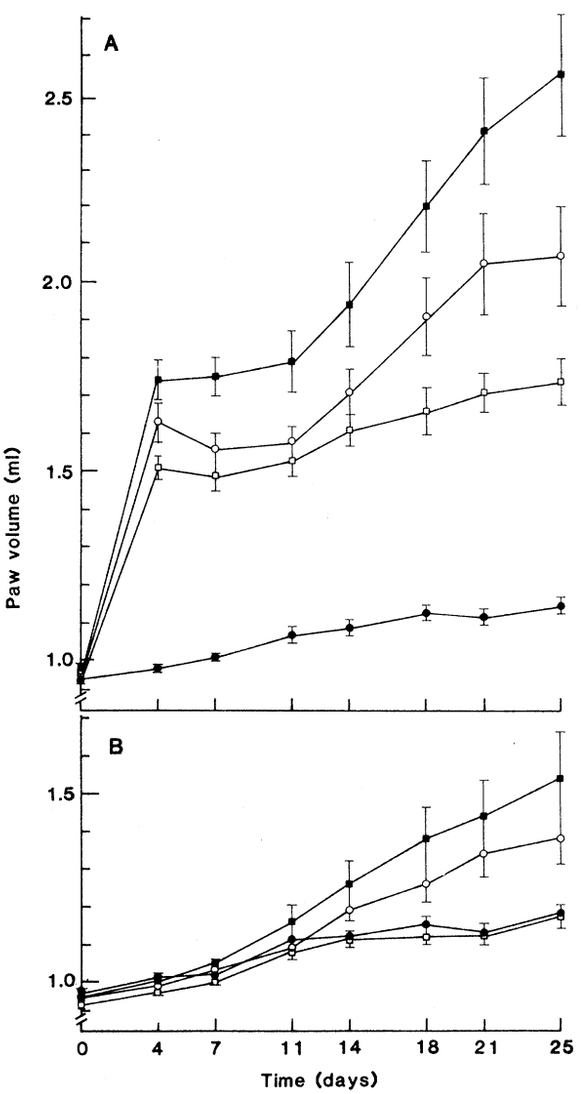

Fig. 1. Effect of orally administered 13-*cis*-retinoic acid on the development of adjuvant arthritis. As a way of following the development of the inflammatory lesions (swelling), the volumes of the adjuvant-injected paw (A) and of the noninjected contralateral paw (B) were measured at intervals of 3 to 4 days by immersion of the paw to the level of the lateral malleolus in a mercury plethysmograph. Means ± the standard errors of the means are plotted for 30 normal rats (●——●); 40 vehicle-treated arthritic rats (■——■); 29 retinoic acid–treated (40 mg/kg) rats (○——○); and 20 retinoic acid–treated (160 mg/kg) rats (□——□). The volume of the adjuvant-injected paw decreased by 29 and 50 percent after 25 days of treatment with 13-*cis*-retinoic acid at doses of 40 and 160 mg/kg, respectively. The corresponding values for the noninjected, contralateral paw were 26 and 61 percent. These decreases were statistically significant at $P < 0.05$ (Student's *t*-test).

13-*cis*-retinoic acid did not affect PGE_2 production by rat synovial cells in vitro. However, we do not know whether 13-*cis*-retinoic acid affected the production of PGE_2 by other cell types in vivo and thereby influenced the development of cell-mediated immunity in the rats with AA.

Retinoids modulate the effects of other mediators. For example, the action of mononuclear cell factor (probably interleukin-1), which stimulates the production of collagenase and PGE_2 by synovial cells in culture, is antagonized by 13-*cis*-retinoic acid (*5*). Retinoids also antagonize the proliferative effect of prolactin on mammary epithelium (*19*) and prevent polypeptide growth factors from inducing expression of phenotypic characteristics of transformed cells in nonneoplastic cells (*20*).

Trentham and Brinckerhoff (*21*) recently have studied the effect of treatment with 13-*cis*-retinoic acid on the development of collagen-induced arthritis in rats. In this model, the administration of retinoic acid was associated with an increase in the severity of the arthritis with no effect on humoral or cellular immune responses to collagen. Synovial cells taken from rats with collagen-induced arthritis also secreted elevated concentrations of collagenase and PGE_2. Oral administration of 13-*cis*-retinoic acid decreased collagenase production by these synovial cells. Thus, despite the fact that 13-*cis*-retinoic acid affected AA and collagen-induced arthritis differently, its effect on collagenase production by synovial cells was the same in both models.

Reasons for the disparate effects of treatment with 13-*cis*-retinoic acid on the severity of arthritis in the two models are unclear, but the dissimilarities may be due to differences in (i) the sex of the rats used (females for collagen arthritis and males for AA), (ii) the dosage of retinoid (rats with collagen arthritis received in the diet only a quarter of the amount of drug administered to rats with AA), or (iii) mediators of the two models of the disease. Other recent experiments have demonstrated that all-*trans*-retinoic acid and corticosteroids at low concentrations ($10^{-10}M$) synergistically inhibit collagenase production by synovial cells in culture (*15*).

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Expression of Streptococcal M Protein in *Escherichia coli*

Abstract. *The structural gene for group A streptococcal M protein, the fibrillar surface molecule enabling the organism to resist phagocytosis, has been cloned into* Escherichia coli. *The molecule produced by* Escherichia coli *is slightly larger than the M protein isolated by solubilization of the streptococcal cell wall, but is similar in size to that secreted by streptococcal protoplast and L forms. Immunologically, the molecule synthesized by* Escherichia coli *has the same type-specific determinants as the streptococcal M protein.*

In developing countries of the tropics and subtropics, rheumatic heart disease is both the major cause of cardiac hospital admissions and the most common form of cardiac damage in school-age children (*1, 2*). Although the exact mechanisms of disease causation are not well understood, it is clear that rheumatic fever, as well as acute nephritis, follows infection with *Streptococcus pyogenes* (group A streptococcus). Fortunately, since group A streptococci are all sensitive to penicillin at this time, prompt treatment with this antibiotic is effective in preventing acute rheumatic fever (*3*). However, for those poorer countries in which these diseases are prevalent, such therapy is both expensive and logistically impractical. Furthermore, it is possible that penicillin-resistant streptococci may arise at any time. For these reasons, the development of a safe and effective vaccine to prevent infection by the group A streptococcus is a desirable objective.

Resistance to group A streptococcal infection depends on the presence of type-specific antibodies to the M protein (*4*), a fibrillar molecule found on the surface of the organism (*5, 6*). The M protein is a major virulence factor of this bacterium because it can impart resistance to attack by the host's phagocytic cells (*7*). Because of its ability to vary the antigenic structure of the M molecule, the group A streptococcus is able to successfully evade the host's type-specific immune response and thus cause recurrent streptococcal disease in man. In addition to a number of nontypable strains, about 70 immunologically distinct M types are now recognized. Despite the fact that antibodies cross-reac-