

AURICULAR CHONDRITIS IN RATS  
An Experimental Model of Relapsing Polychondritis  
Induced with Type II Collagen\*

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Relapsing polychondritis is an inflammatory disease of connective tissue characterized by the destruction of hyaline and elastic cartilages. Although the etiology of this disease is unknown, the demonstration of anti-cartilage antibodies in the sera of patients with this disorder suggests an autoimmune mechanism (1, 2). Recently, Foidart et al. (3) have reported an association between relapsing polychondritis and autoimmunity to type II collagen, the major collagen type in cartilage. This finding was of particular interest because we (4) and others (5, 6) have reported that rats and mice (7), after sensitization with purified native type II collagen, developed inflammatory polyarthritis that was closely associated with an intense immune response to type II collagen (4-6).

We have recently discovered that some rats sensitized with type II collagen also develop inflammatory ear lesions (8) characterized by an intense, destructive chondritis resembling the histopathologic process seen in human relapsing polychondritis. Described here are our findings, which closely associate the presence of these experimental lesions with the immune response to native type II collagen. These observations suggest that collagen-induced chondritis in rats might serve as an experimental model for human relapsing polychondritis.

### Materials and Methods

*Animals.* Outbred female Wistar and inbred Wistar-Lewis rats weighing 100-125 g were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. and fed laboratory chow and water ad libitum. Wistar-Lewis rats were provided through the courtesy of the National Cancer Institute.

*Preparation of Collagen.* Native bovine and rat type II collagens were solubilized by limited pepsin digestion of fetal calf cartilage and a rat chondrosarcoma tumor, respectively, and purified as described in a previous report (4). The rat chondrosarcoma tumor was a gift from Dr. George Martin of the National Institute of Dental Research. The purity of these collagens was determined by amino acid analysis, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and uronic acid analysis.

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*Immunization of Rats.* Rats were injected intradermally in a hind footpad with 200  $\mu\text{g}$  of bovine type II collagen in an emulsion prepared by emulsifying equal volumes of incomplete Freund's adjuvant and bovine type II collagen in 0.1 M acetic acid (4 mg/ml). A second injection of a similar emulsion was given into the tail 7 d later. Adjuvant arthritis was induced by injecting rats at the base of the tail with 250  $\mu\text{g}$  of ground *Mycobacteria hominis* suspended in mineral oil.

*Histological Studies.* Tissue for light microscopy was processed by a standard method and stained with hematoxylin and eosin. Tissue for electron microscopy studies was fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.2, followed by post-fixation in cacodylate-buffered osmium tetroxide, embedded in epoxy resin, sectioned, stained with uranyl acetate and lead citrate, and examined as previously described (9). Immunofluorescence studies were performed on snap-frozen tissue sections by a standard method using fluorescein-conjugated goat anti-rat IgG, C3, and fibrinogen. Antisera were obtained from N. L. Cappel Laboratories, Cochranville, Pa. Tissue for immunofluorescence studies was taken from an area adjacent to the samples used for electron microscopy.

*Antibody Assay.* Antibody levels were determined by an enzyme-linked immunosorbent assay (ELISA). Collagens were adsorbed to polystyrene cuvettes (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) for 16 h at 4°C in 0.4 M potassium phosphate buffer, pH 7.6 (25  $\mu\text{g}/\text{ml}$ ). The cuvettes were washed with 0.15 M NaCl (saline) supplemented with 0.05% Tween 20, incubated for 1 h with 0.1 M Tris-HCl-buffered saline, pH 7.5, containing 1% bovine serum albumin and washed. Sera, diluted at 1:1,000 with 1% bovine serum albumin-Tris-saline, supplemented with 0.05% Triton X-100, were added to the cuvettes and incubated for 16 h at 4°C. After washing, peroxidase-conjugated goat anti-rat IgG or rabbit anti-mouse IgM antisera (N. L. Cappel Laboratories) were added at predetermined dilutions. 4 h later, after washing, 52 mM 6-amino salicylic acid dissolved in distilled water was added. The colorimetric reaction was read 30 min later at 450 nm by using a Gilford EIA system (Gilford Instrument Laboratories, Inc.) and expressed as absorbance.

## Results

*Clinical Features.* Bilateral auricular chondritis was observed in 12 of 88 outbred Wistar rats (13.6%) sensitized with native bovine type II collagen. 8 of the 12 rats with ear lesions also developed collagen-induced arthritis, which occurred in a total of 68 of the 88 rats immunized (77.3%). Ear lesions appeared significantly later between 27 and 76 d after injection as compared with arthritis, which appeared between days 10 and 29 (mean values,  $49.8 \pm 5.2$  SE and  $13.4 \pm 0.4$ , respectively,  $P < 0.001$ ). In contrast, ear lesions were not seen in 85 inbred Wistar-Lewis rats injected in an identical manner and observed for 8 wk. Nonetheless, these rats were susceptible to the induction of arthritis and 75 (88.2%) became arthritic.

Auricular chondritis was first manifested as discrete erythematous macules that increased in number and size, coalescing to produce diffuse erythema and induration of the ears (Fig. 1). Over the next 8 wk, the swelling gradually subsided, leaving the ears thickened and stiff.

The clinical appearance and course of chondritis induced by type II collagen were distinct from the ear lesions observed in rats with adjuvant arthritis. Ear nodules induced by mycobacteria were seen in each of 20 rats with adjuvant arthritis and appeared between days 11 and 17 ( $13.9 \pm 0.4$  SE), often coincident with the onset of arthritis. These nodules did not result in diffuse auricular disease and healed without scarring.

*Histologic Features.* Auricular tissue obtained from rats with ear lesions revealed multifocal destruction of the cartilage ear plate and perichondrium by an inflammatory process. Fig. 2 represents a 14-d-old lesion. The cellular infiltrate consisted primarily of histiocytes and chondroblasts plus a lesser number of lymphocytes and

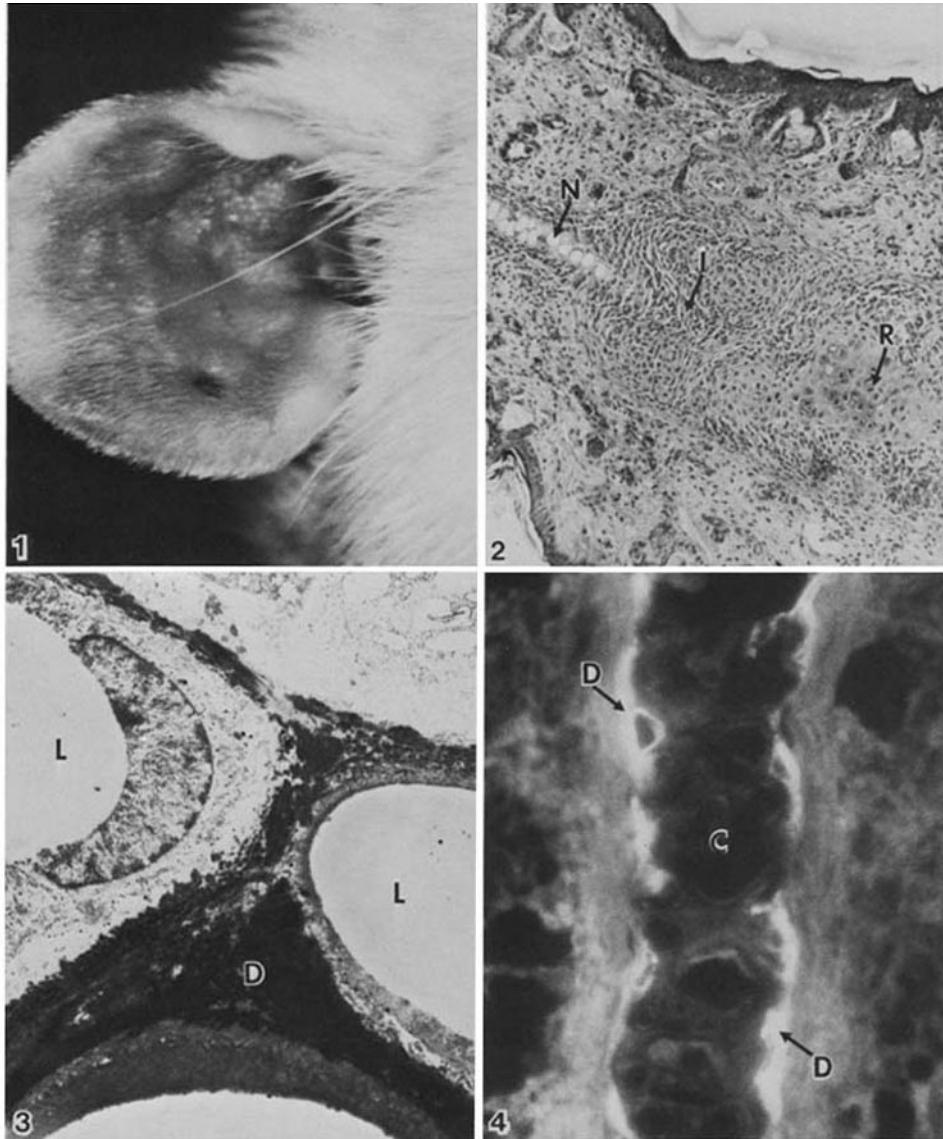


FIG. 1. The clinical appearance of a 14-d-old ear lesion of a rat with collagen-induced chondritis. The ear is erythematous and thickened, and a small area of normal tissue is seen near the periphery of the ear.

FIG. 2. Histologic appearance of a 14-d-old chondritic ear lesion. An area of normal cartilage (N) is seen adjacent to a site of active inflammation (I). Also shown is a focus of regenerating chondrocytes (R).  $\times 100$ .

FIG. 3. An electron photomicrograph of ear cartilage obtained from a clinically normal area 7 d after the onset of chondritis. A large electron-dense deposit (D) is seen near the surface of a chondrocyte. Large lipid vacuoles (L) are seen within the chondrocytes.  $\times 4,000$ .

FIG. 4. Ear cartilage after staining with fluorescein-conjugated goat anti-rat IgG. Tissue was obtained adjacent to that studied in Fig. 3. Deposits of IgG (D) are shown within the matrix of the auricular cartilage (C).  $\times 400$ .

TABLE I  
*IgG Antibody to Type II Collagen Measured by ELISA\**

Rats with	Number of sera studied	Absorbance at 450 nm	
		Bovine type II collagen	Rat type II collagen
Chondritis	8	1.096 ± 0.086‡	0.927 ± 0.105
Arthritis without chondritis	10	0.719 ± 0.075§	0.710 ± 0.095
Without arthritis or chondritis	6	0.083 ± 0.006	Not done

\* Antibody reactive with bovine and rat type II collagens were measured by ELISA using sera from rats sensitized with bovine type II collagen.

‡ All sera were studied at a dilution of 1:1,000 and results are expressed as mean absorbance for each group ± SE. Sera from normal rats gave absorbance values of 0.018 and 0.041, respectively, for cuvettes coated with bovine or rat native type II collagen.

§ Significant at  $P < 0.01$  (student's  $t$  test) compared with rats with chondritis.

neutrophils. A prominent focus of chondrocyte regeneration was also seen. Ear lesions examined at 72 h frequently appeared near small blood vessels that penetrated the cartilage plate and contained more neutrophils. The ears of eight rats with collagen-induced arthritis, but no gross evidence of ear lesions, were histologically normal, though sampled at comparable times.

Ear nodules induced by injecting mycobacteria in oil were histologically distinct from those induced by type II collagen. Regardless of age or severity, the ear lesions examined from rats with adjuvant arthritis completely spared the elastic cartilage as originally reported (10).

*Electron Microscopy and Immunofluorescence Studies.* Electron-dense deposits were prominent in the clinically normal auricular cartilage of each of two rats studied with collagen-induced chondritis and were not observed in nonsensitized controls or two arthritic rats without chondritis biopsied 60 d after immunization. The deposits were seen near the surface of chondrocytes (Fig. 3) and were irregular in shape and size. It is improbable that these deposits were an artifact of inflammation because sections cut from the same blocks and examined by light microscopy revealed no inflammation.

Positive immunofluorescence with anti-rat IgG (Fig. 4) and C3 antisera were observed in the matrix below the chondrium adjacent to the chondrocytes and corresponded to the location of the electron-dense deposits. The same areas gave negative fluorescence with anti-rat fibrinogen antisera.

*Studies of Humoral Immunity.* Sera were obtained from eight rats within 1 wk of the appearance of chondritis and studied by ELISA. Control sera were obtained at comparable intervals after immunization from 10 rats with arthritis but without ear lesions, and 6 rats also sensitized with type II collagen, but without arthritis or chondritis.

Sera from rats with chondritis (Table I) contained significantly greater amounts of IgG reactive with bovine type II collagen ( $P < 0.01$ ) than matched immunized controls. Additional studies revealed a high degree of cross-reactivity between IgG and rat type II collagen. IgM antibody reactive with type II collagens was not detected, most likely reflecting the long interval between sensitization and the collection of sera. IgM and IgG antibodies to rat type I collagen were not detectable by ELISA.

### Discussion

We have shown for the first time that 14% of outbred Wistar rats injected with bovine native type II collagen developed auricular chondritis resembling that of

human relapsing polychondritis. These lesions appeared long after sensitization, occurred independently of collagen-induced arthritis, and were distinct from the ear lesions of adjuvant arthritis. Chondritis was characterized by the destruction of elastic cartilage by an inflammatory process. Early lesions contained frequent neutrophils that were followed by the appearance of histiocytes and regenerating chondroblasts. Electron-dense deposits were found near the surface of chondrocytes that corresponded with the location of immunofluorescence using antisera to rat IgG and C3. This finding was of interest because both IgG and C3 have been demonstrated in the cartilage of patients with relapsing polychondritis (11-13). Further, as in human disease (3), IgG antibody to native type II collagen was found in the sera of rats with active chondritis.

These findings suggest that collagen-induced chondritis in rats is mediated by IgG antibody and complement. It is most probable that IgG demonstrated in the auricular cartilage was reactive with type II collagen because high levels of antibody cross-reactive with native rat type II collagen were demonstrated in the sera of rats with ear lesions. These findings, however, do not explain whether IgG deposited in elastic cartilage was trapped immune complex or antibody bound to matrix type II collagen. Furthermore, the potential importance of cell-mediated immunity and antibody-dependent cytotoxicity cannot be excluded as mechanisms of injury, although the predominance of neutrophils in early lesions does not suggest these as primary events. Additional studies are needed to determine the importance cell-mediated immunity in this model and to define the specificity of IgG localized in tissue lesions.

The late appearance of ear lesions was of interest because we have shown previously (4) that antibody to type II collagen appeared soon after immunization, coincident with the onset of arthritis. This observation might be explained by the structural differences in cartilage of the ear and diarthroidal joints. Cartilage in both sites is avascular and derives nutrients by passive diffusion. Elastic cartilage, in comparison with hyaline cartilage, lacks a synovial lining and has a thicker perichondrium, features that might impede deposition of IgG and complement or retard the entry of neutrophils. IgG might accumulate slowly until a critical amount is reached sufficient to initiate inflammation. This would make the appearance of ear lesions dependent upon time, levels of circulating IgG, and the rate of loss of IgG from the matrix. The presence of IgG in histologically normal auricular cartilage obtained adjacent to lesions is consistent with this hypothesis. Alternatively, a quantitative difference in IgG subclasses or a shift in epitope specificity might be important and could occur long after sensitization. This last possibility is suggested indirectly by our observation that only outbred rats developed ear lesions. Inbred Wistar-Lewis and some outbred Wistar rats might be genetically restricted and unresponsive to a certain epitope(s) associated with chondritis.

### Summary

Outbred Wistar rats immunized with native type II collagen developed ear lesions resembling those of human relapsing chondritis. As in human disease, these lesions were characterized by intense chondritis, positive immunofluorescence reactions to IgG and C3, and circulating IgG reactive with native type II collagen. Furthermore, electron-dense deposits were seen near the surface of chondrocytes and corresponded with deposits of IgG and C3. These observations suggest a causal relation between humoral immunity to type II collagen and auricular chondritis in the rat and support

the hypothesis that human relapsing polychondritis is an autoimmune disease mediated by immunity to type II collagen.

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