Brief Definitive Report

INDUCTION OF ARTHRITIS IN MONKEYS BY IMMUNIZATION WITH TYPE II COLLAGEN

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Collagen-induced arthritis (CIA) is an inflammatory arthropathy induced by immunizing rodents with type II collagen, a major protein of hyaline cartilage (1). CIA is characterized by proliferative synovitis that erodes the adjacent cartilage, ultimately producing joint destruction and ankylosis (2). It shares important clinical, histological, genetic, and immunologic features with human rheumatoid arthritis (RA) (3). However, there are significant differences between CIA and RA and the relevancy of CIA to human arthritis has been debated (4). Among the limitations of CIA as a model was that it could be induced only in rodents and not in other commonly used laboratory animals, including guinea pigs and rabbits. However, it was recently reported that squirrel monkeys are also susceptible to CIA (5). This means that CIA is not a disease peculiar to certain rodents but can also be induced in primates. We report here that rhesus and cynomolgus monkeys also develop polyarthritis after immunization with type II collagen.

Materials and Methods

Animals. Three adult cynomolgus monkeys, *Macaca fascicularis*, weighing 5–7 kg, and five rhesus monkeys, *Macaca mulatta*, were used in this study. All monkeys were female except monkey number 88 who was a castrated male monkey. They were housed individually, fed a commercial monkey diet supplemented with fresh fruits, and maintained in a 12-h light/12-h dark cycle. Room air temperature was kept at 72 ± 2°F and at 50 ± 20% relative humidity. Air was not recirculated and was exchanged 12–15 times each hour.

Antigens. Native type II and type I collagens were prepared from fetal bovine cartilage and skin, respectively, as previously described (1). Collagen fragments were prepared by cyanogen bromide digestion of constituent α chains and purification of the resulting peptides. Each peptide was renatured to its native triple helical conformation by stepwise cooling as previously described in detail (6).

Immunizations. Native collagen was dissolved in 0.1 N acetic acid at a concentration of 2 mg/ml by stirring overnight at 4°C. The collagen solution was then emulsified with Freund's adjuvant using an homogenizer (VirTis Co., Inc., Gardiner, NY). Two cynomolgus monkeys were each immunized intramuscularly in the posterior thigh with 1 mg of native type II collagen in CFA. Booster immunizations were given with 1 mg of collagen emulsified in IFA at intervals as shown in Fig. 1. An unimmunized monkey of the same species served as a control.

Three rhesus monkeys were also immunized with 2 mg of type II collagen in CFA and

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boosted with 1 mg in IFA at monthly intervals for 2 mo. A final booster was given after 5 mo. Two monkeys of the same species were used as controls: one was immunized with type I collagen and another was not immunized.

Clinical Observations. The monkeys were observed regularly for signs of arthritis. Their weights were recorded and blood was obtained for hemograms, antibody determination, and erythrocyte sedimentation rate. The presence of arthritis was confirmed by radiological examination.

Measurement of Antibody Levels. Serum antibody titers were measured by modification of an ELISA described previously (7). ELISA-plates were coated with intact collagen or cyanogen bromide-derived peptides 8, 10, 11, and 12 at a concentration of 5 μg/ml. Assay temperature was maintained at 4°C to prevent denaturation of the renatured peptides. Sera for analysis were diluted in 0.15 M NaCl, 0.1 M Tris-HCl, pH 7.4, containing 0.05% Tween 20 (PBS-Tween) and were incubated in the ELISA plates overnight. The plates were washed with PBS-Tween and incubated with peroxidase-conjugated goat anti-monkey IgG. Plates were again washed and developed with orthophenylenediamine dissolved in citrate-phosphate buffer, pH 5.5, containing H_2O_2. After 1 h the absorbance of each well was measured at 490 nm using an automated reader (model 580; Dynatech Laboratories, Inc., Alexandria, VA).

Histology. Specimens for histologic examination were fixed in cold 95% ethanol, decalcified in cold 5% ethylene diamine tetraacetic acid solution, pH 7.4, and prepared for staining using the technique described by Sainte-Marie (8). Sections were cut at 7 μm and stained by hematoxylin and eosin.

Results

Clinical Observations. The earliest change noted after immunization was anorexia developing as early as 10 d after primary immunization. All three rhesus monkeys lost weight by day 59 while the control monkeys had a moderate gain (Fig. 1). Anorexia in some monkeys was severe enough to warrant daily feeding by stomach tube. All of the type II collagen-immunized monkeys developed arthritis. The interphalangeal joints and wrists became swollen and warm to the touch. Some joints exhibited distinct fine crepitation. The presence of arthritis was confirmed by radiography (Fig. 2). The arthritis in the wrists persisted and resulted in marked limitation of motion (Fig. 3). Acute episodes of arthritis and anorexia tended to occur ~10–14 d after booster and lasted for 2 wk. Monkeys lost between 32 and 43% of their body weight before recovery began. The ability of a booster immunization to cause a flair of arthritis was variable. During the acute episodes monkeys became depressed and less mobile. Erythrocyte sedimentation rates were elevated (32–52 mm). Two monkeys

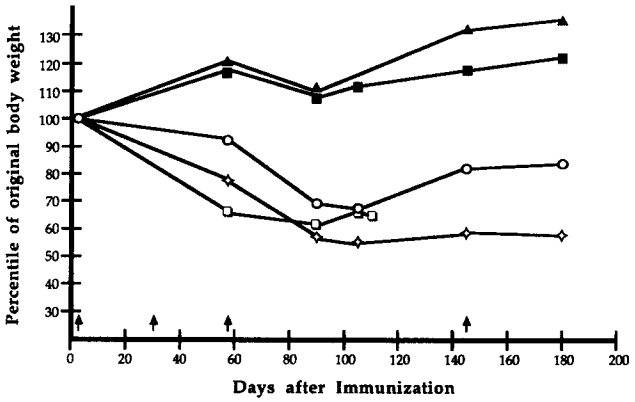

FIGURE 1. Body weights of experimental animals monkey No. 1122 (o); monkey No. 411 (✧); monkey No. 419 (□). These monkeys were immunized with type II collagen; monkey no. 416 (■) was not immunized; monkey 88 (▲) was immunized with type I collagen; Monkeys were initially immunized with 1 mg of type II collagen in CFA adjuvant. Booster (↑) were given using 1 mg collagen in IFA. Experiment on monkey no. 419 was terminated at day 108.

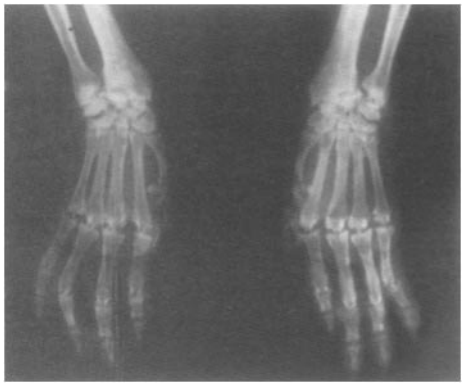

FIGURE 2. Radiograph of the wrists and hands of a monkey 3 mo after immunization with type II collagen. There is soft tissue swelling and marked destructive changes in both wrists. Several metacarpophalangeal and interphalangeal joints are narrowed with erosions.

refused to bear weight on their legs and sat in the back corner of their cages. This resulted in contracture of the flexor tendons of the legs which did not resolve.

Discomfort was judged to be sufficiently severe enough to require therapy and aspirin was given by gavage in a dosage of 25 mg/kg/d. In addition, injectable analgesics (butorphanol, nalbuphine) were used on some occasions. Most of the monkeys improved after 100 d. There was increase in body weight, mobility, and a return of normal disposition. The most severely affected monkey, however, had to be euthanized at day 108 because of severe inanition.

Pathologic Changes. At autopsy, the connective tissue of the arthritic knees, wrists, metacarpophalangeal, and interphalangeal joints were tightly adhered to the joint capsules. The cartilage surfaces were rough and eroded (Fig. 4). In contrast, both the unimmunized and type I collagen–immunized monkeys had freely movable joints with smooth cartilage surfaces. By histologic examination, synovial hypertrophy was detected along with cartilage destruction. Some neutrophils were seen on surfaces of the eroded articular cartilage. The synovium was hypertrophic and there was lymphocyte infiltration around blood vessels. (Fig. 5). In some areas subchondral bone was destroyed by granulation tissue. A mild proteinaceous exudate including a few neutrophils was seen in the joint lumen.

Immune Response to Collagen. All of the monkeys immunized with type II collagen developed high levels of circulating antibody specific for the immunizing antigen

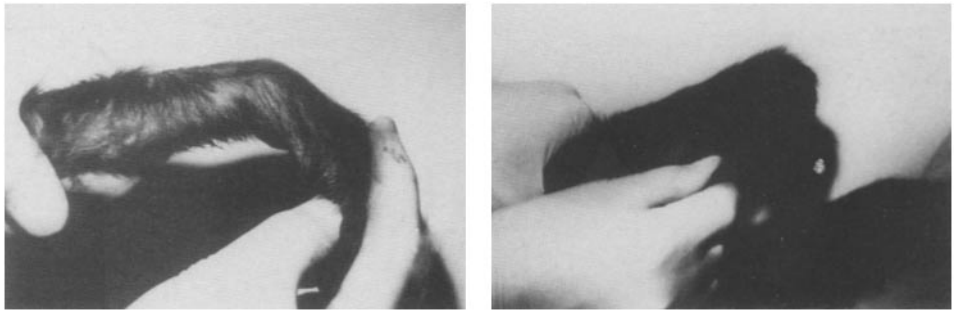

FIGURE 3. Photograph of the wrist of a monkey immunized 3 mo previously with type II collagen showing restricted flexion (*left*) the wrist of a control monkey (*right*) is shown for comparison.

FIGURE 4. Cartilage surface of knee joints from type II collagen immunized (*right*) and control animal (*left*) at autopsy. The control joint has a smooth cartilage surface in contrast to the rough and eroded cartilage surface of the arthritis joint.

(Table I). When antibody levels to fragments of collagen were measured, there was substantial heterogeneity in the response (Table I). However, all monkeys responded strongly to cyanogen bromide peptide 11, which has previously been shown to contain an epitope arthritogenic for DBA/1 mice (6). This was in fact the only peptide with which all immune sera reacted strongly.

Discussion

Cathcart and colleagues (5) have shown that squirrel monkeys will also develop arthritis after immunization with type II collagen, and we have now shown that a primate even more closely related to man will also develop arthritis after immunization with type II collagen. In addition, the arthritis we observed was characterized by cartilage and bone erosions quite similar to those seen in human rheumatoid arthritis.

The disease we observed differed in some respects from that reported in squirrel monkeys. Its onset was insidious, with constitutional symptoms appearing first. Only later did joint inflammation become clearly evident. In some individual monkeys the disease was relapsing or could at least be induced to flare by reinjecting the animal with type II collagen. In squirrel monkeys the disease was abrupt in onset with

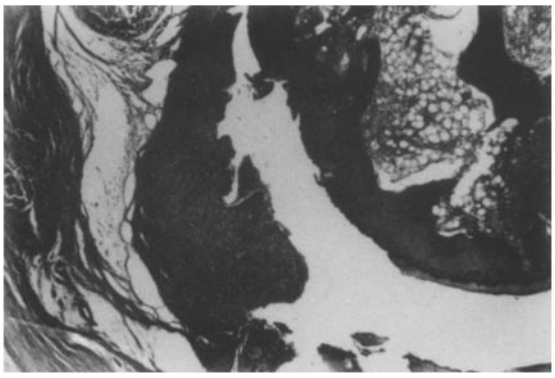

FIGURE 5. Histopathology of a middle finger from a monkey 4 mo after immunization (× 320). There is synovial hypertrophy with marked infiltration of the subsynovial tissue with inflammatory cells. The cartilage appears irregular and marginal erosion is present. On higher power examination most of the cells appear to be lymphocytes but many neutrophils were also seen.

TABLE I
Antibody Titer to Type II Collagen Measured by ELISA

Monkey number	Test antigen	Absorption at 490 nm at Sera dilution of: 1/100 BIIn	1/500 BIn
416	Control	0.048	–
88	Type I*	0.028	0.33
419	Type II‡	1.500	–
411	Type II‡	0.591	–
1120	Type II‡	0.522	–

* Animals were immunized with native bovine type II collagen.
‡ Animals were immunized with native bovine type I collagen.

very rapid progression. In both squirrel monkeys and our animals severe inanition occurred. The basis for this is not certain. In one case it was sufficiently severe to necessitate termination of the experiment with euthanasia of the monkey. This inanition may have been in part due to the inability to grasp food but this seems an inadequate explanation. At autopsy, no gastrointestinal lesion was identified. The monkeys were, however, noted to have lesions of the middle and inner ear. Such lesions have previously been reported in rodents (9) and may have contributed to nausea, vertigo, and anorexia in the monkey.

Immune responses to collagens were elicited in all immunized monkeys. The antibody level to type II collagen fragments were heterogeneous; however, all of the antisera responded strongly to CB-peptide 11 which contains an epitope arthrogenic for DBA/1 mice (6). Though our data suggest the CB-11 peptide could be an important arthrogenic epitope in monkeys, further experiments using individual CB peptide fragments as immunizing antigens are needed to clarify this point.

Summary

Immunization of two cynomolugus and three rhesus monkeys with purified type II collagen resulted in the development of polyarthritis. Arthritis first became clini-

TABLE II
Antibody Against Cyanogen Bromide Peptides of Type II Collagen 2 mo after Immunization

Group	Animal number	Dilution	Absorbance at 490 nm CB8*	CB10	CB11	CB12
Control	416	1:75	0.066	0.106	0.101	0.119
BIn	88	1:500	0.045	0.051	0.068	0.091
BIIn	411	1:500	0.081	0.358	0.407	0.117
BIIn	419	1:500	0.091	0.130	0.351	0.850
BIIn	1121	1:400	0.180	0.297	0.389	0.115

* Cyanogen bromide peptides were obtained by cleaving purified chick type II collagen with cyanogen bromide (CB).
‡*BIn, animal immunized with native bovine type I collagen; BIIn, animals immunized with native bovine type II collagen.

cally apparent 7 wk after primary immunization and persisted for 16 mo. Radiologic examination of the limbs demonstrated soft tissue swelling with severe joint destruction including loss of cartilage and bone. Involved joints eventually became ankylosed with permanent loss of some motion. All of the monkeys developed a response to the immunizing collagen as determined by ELISA of serum for antibodies. Arthritis was associated with weight loss and constitutional symptoms, including lethargy and refusal to eat. One monkey became so debilitated that it was necessary to euthanize it. Histologic examination of the joints showed synovial hypertrophy with pannus formation. A control monkey immunized with type I collagen suffered no apparent ill effects.

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