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Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats

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SUMMARY

Rats were per-gastrically dosed by gavage with collagen type II (CII) in solution and were shown, as a result, to be subsequently resistant to the induction of disease by CII administered parenterally in Freund's incomplete adjuvant. Disease onset was delayed and the severity was reduced. There was no concomitant reduction in the systemic antibody response to CII. Gavage with CII did not in itself induce systemic anti-CII antibodies and did not cause symptoms of arthritis.

The results indicate that materials specifically cross-reactive with autoantigens can be absorbed through the gut and can modify susceptibility to autoimmune disease.

Keywords collagen type II arthritis mucosal immunity

INTRODUCTION

Rats develop polyarthritis after being injected with solubilized Collagen type II (CII) emulsified in Freund's Incomplete Adjuvant (FIA) (Trentham, Townes & Kang, 1977). The susceptibility to disease is genetically controlled and there is compelling evidence that autoimmunity against articular CII is invovled in its pathogenesis (Stuart, Townes & Kang, 1984, review).

The physical form of the CII and the way in which it is administered are crucial to the development of disease. If soluble collagen is not used or if it is not presented in FIA then disease will not be induced. Further, rats will become refractory to disease induction if they are exposed to either soluble collagen injected intravenously (Staines *et al.*, 1981), or to collagen-coupled spleen cells (Schoen, Green & Trentham, 1982) or to polymerized collagen (Henderson & Staines, submitted). Likewise, exposure to antibody against collagen may have a similar suppressive effect (Staines *et al.*, 1981). Such parenteral treatments will modify the immune system in such a way that self tolerance to CII is not then perturbable by arthritogenic CII.

In view of the fact that much of the antigenic stimulation in the natural environment is received through the mucous membranes we have investigated the effects of per-gastric exposure to collagen on the development of arthritis. We report here that this can modify susceptibility to severe arthritic disease in rats.

MATERIALS AND METHODS

Animals. Inbred 3- to 4-month old male (240–280 g) WA/KIR rats were obtained from The Kennedy Institute, Hammersmith, UK and were maintained in solid-bottomed plastic cages with woodshavings as litter.

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Induction and assessment of arthritis. Collagen was enzymically solubilized and disease induced by injecting rats (in groups of at least five animals each) with 0·5 mg CII intradermally in FIA as described previously (Staines *et al.*, 1981).

Animals were regularly weighed and assessed clinically. The severity of arthritis in each paw was scored on a scale of 0 to 4 based on increasing periarticular erythema and swelling of soft and hard tissues. An aggregate arthritic index (maximum 16) was derived for each animal and a group aggregate derived expressed as a percentage of the maximum possible score for each group. This permitted groups of different sizes to be compared directly.

Serology of anti-CII antibodies. Anti-CII antibodies were titrated in an enzyme-linked immunosorbent assay (ELISA) as described elsewhere (Staines *et al.*, 1981) except that the sera were diluted in phosphate buffered saline, pH 7·2, containing 0·1% Tween-20 (Sigma Chemical Co., Poole, Dorset, UK) and 1·0% Casein and the *o*-phenylenediamine (British Drug Houses, Dagenham, Essex, UK) was used at 1 mg/ml.

Gavage of Rats. CII was dissolved in 0·1 M (ethanoic) and administered with a plastic tipped 0·63 mm diam cannula via the oesophagus directly into the stomach. Animals received single doses on 5 consecutive days (up to and including the day of challenge) of 2·5 or 25 μg CII/g body weight.

RESULTS

The onset of disease was delayed and the severity of disease was reduced in animals that were dosed pergastrically with CII before the arthritogenic challenge (Fig. 1). Two different doses of CII were administered by gavage and it was in animals receiving the lower dose that the greatest suppression of disease was seen. In these animals, first signs of arthritis were not apparent until 18 days after challenge and the disease did not reach its maximum severity, which was similar finally to the control animals, until 5 weeks after challenge. Less suppression was seen in animals receiving the higher dose of CII intragastrically. In these the first signs of disease appeared on day 11 and maximum severity was reached by day 18. In comparison, control animals that received only ethanoic acid in the gavage also showed initial symptoms on day 11 but progressed more rapidly to

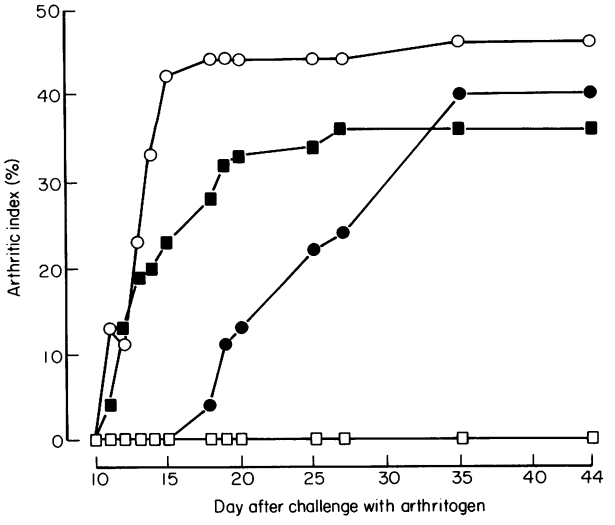

Fig. 1. Effects of per-gastric administration of soluble CII on induced arthritis. Animals were dosed daily with 25 μg CII/g (■) or 2·5 μg CII/g body weight (●) by gavage for 5 days and challenged with CII in FIA injected intradermally on day 5. Control animals were gavaged with 25 μg CII/g body weight and not challenged (□) or were challenged after receiving only ethanic acid in the gavage (○). Arthritic index is the group aggregate (5 animals/group) based on the scoring system described in the text.

Table 1. Serum levels of anti-CII antibodies in rats dosed by gavage with soluble CII before parenteral challenge with arthritogenic CII in FIA

CII gavage μg CII/gm body weight per dose	Parenteral challenge with arthritogenic CII	Serum anti-CII titre in ELISA (log_{10} mean ± s.d.) Days after immunization 0	7	14	20	27	35
25	+	<1·0	1·38±0·48	4·14±0·20	4·10±0·12	4·57±0·36	3·84±0·10
25	−	<1·0	<1·0	<1·0	<1·0	<1·0	<1·0
2·5	+	<1·0	1·19±0·31	3·98±0·43	4·45±0·11	4·15±0·21	4·00±0·16
0	+	<1·0	1·47±0·17	4·12±0·08	4·57±0·34	4·17±0·07	4·15±0·28

the maximum disease state by day 14. This was the same as the previously reported disease kinetics in this strain (Staines *et al.*, 1981; Henderson *et al.*, 1984).

The administration of CII by gavage was not in itself adequate, at the doses used, to cause any clinical disease, nor did it induce a detectable serum anti-CII antibody response.

Whereas the collagen gavage suppressed the subsequent induction of disease it had no effects on the antibody responses of the rats to the arthritogenic CII challenge. There was a distinct dissociation of these two aspects of their reactivity to collagen (Table 1).

The results of this representative experiment have been confirmed in three subsequent series. In each, the onset of disease was delayed by gavaging the animals with 2·5 μg/g CII; the median time of onset being significantly increased ($P < 0{\cdot}05$, Kolmogrov-Smirnov test) between the proportion of healthy animals in the two sets of animals between days 13 and 25 inclusive (Fig. 2). As well as

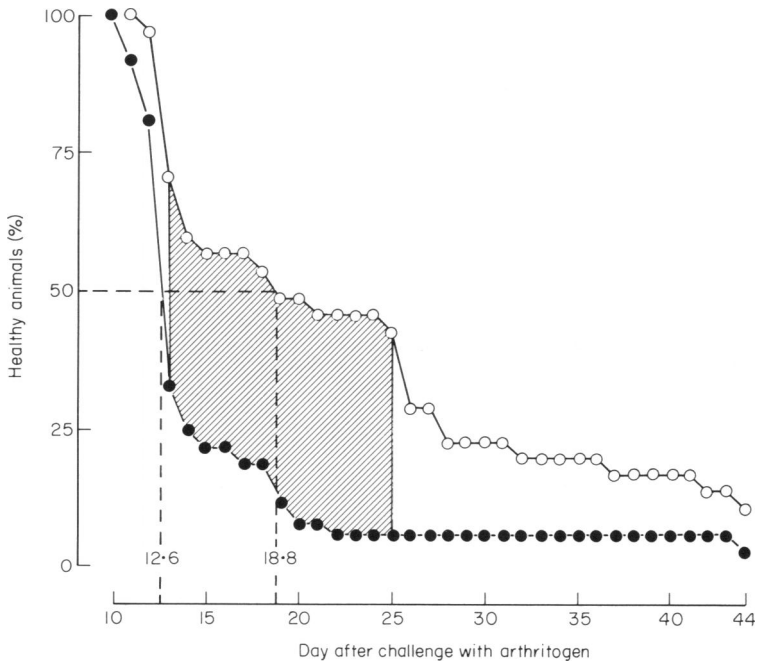

Fig. 2. Effects of per-gastric administration of CII on the time of onset of arthritis. Data pooled from three experiments conducted to same protocol. Animals were dosed daily with 2·5 μg CII/g body weight (35 animals total) (○) or ethanoic acid (36 animals total) (●) by gavage for 5 days and challenged with arthritogen on the fifth day. Shaded area shows period during which disease incidence in the two groups was significantly different ($P < 0{\cdot}05$, Kolmogrov-Smirnov test).

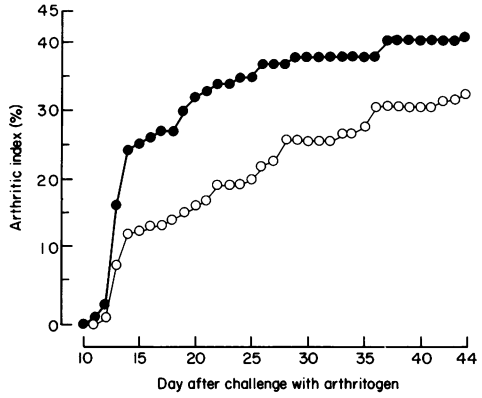

Fig. 3. Effects of per-gastric administration of CII on the severity of arthritis. Symbols and details as Fig. 1. Arthritic index was significantly different in the two groups between days 13–28 inclusive ($P < 0{\cdot}01$, Mann-Whitney U test.

delaying the onset of arthritis, the treatment reduced significantly ($P < 0{\cdot}01$, Man Whitney U test) the severity of disease between days 13 and 28 inclusive (Fig. 3).

Although gastric exposure to collagen had pronounced effects on disease it did not, as previously, alter the quantitative antibody response to collagen. No direct correlation between disease severity and antibody titres was recorded (Fig 4).

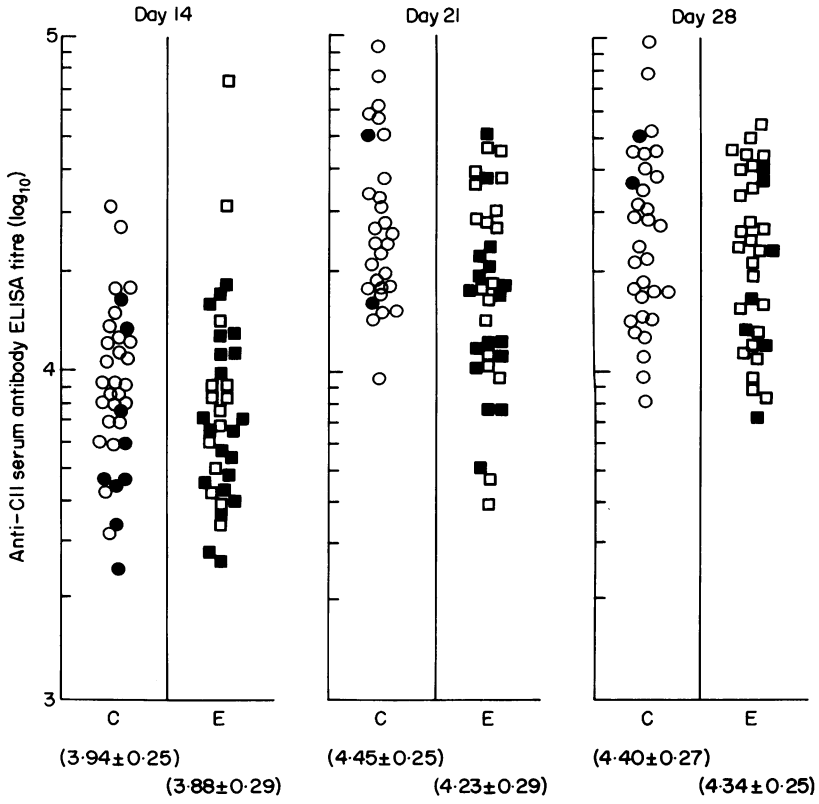

Fig. 4. Effects of per-gastric administration on serum anti-CII antibody titres. Details as Fig. 1. Experimental animals (E) received CII and control animals (C) received ethanoic acid by gavage. Closed symbols represent animals without clinical signs of disease and numbers below each column represent the mean ± standard deviation of the data group.

DISCUSSION

Exposure of rats to collagen through the gut rendered them refractory to polyarthritis that could normally be induced by the parenteral injection of arthritogenic CII. Onset of induced disease was delayed and the severity of disease reduced.

It might be assumed from previous studies of this disease that the pathology is a result of the substantial immunity against CII, which, because of the similarity between the immunizing CII and autologous CII, leads to cross-reactive immunity which is directed against the collagen in joint tissues. In this context it was significant that in these experiments the depression of arthritis was not accompanied by a significant change in the systemic antibody response to CII. If it is correct that there is a direct relationship between immunity and pathology it could be argued that in this case it was undetected qualitative rather than quantitative changes in the immune response that were involved in suppressing the disease. The dissociation between disease development and the antibody response has been seen in other situations where the arthritic disease has been modified, for example, by transferring immune lymphoid cells to naive rats without, however, modifying the antibody response to parenterally administered CII (Burrai *et al*, 1985).

Some evidence for a dose-response effect was found although more extensive experiments are required to establish this. It was found that dividing a given dose into several gavage administrations was more effective at depressing arthritis than the same dose administered in one bolus (data not shown). Similarly, in other experiments (not shown) there were indications that the sex and age of animals influenced the expression of the suppressive effects of pergastrically administered CII.

Most holistic models of autoimmunity attempt to accommodate the influence of enivronment upon the aetiology of disease. Naturally, the mucous membranes of the body must be the portal of entry for the bulk of the antigenic stimulation received by the mammalian immune system and as such presumably hold one key to understanding environmental influences on the initiation of autoimmune disease. It has been shown that ingestion of food allergens may exacerbate rheumatoid arthritis in specifically allergic individuals (Walport, Parke & Hughes, 1982; Little, Stewart & Fennessy, 1983). Feeding of rabbits with cow's milk can induce polyarthritis (Coombs & Oldham, 1981) and appropriate dietary changes in pigs may induce arthritis (Mansson *et al.*, 1971) or influence the course of lupus disease in mice (Levy & Morrow, 1983). Ingestion of dietary analogues of retinoic acid and polyunsaturated fatty acids will modify CII-induced arthritis itself in rats (Trentham & Brinckerhoff, 1982; Prickett, Trentham & Robinson, 1984) and there is evidence that the incorporation of similar compounds in the diet can influence the progression of multiple sclerosis in man and experimental allergic encephalomyelitis in rats (Mertin, 1980).

The existing evidence that diet influences autoimmune disease is primarily concerned with immunologically non-specific effects. The present study is the first to report an immunologically specific effect in the sense that the modifying agent administered pergastrically is the same as the antigen that, when injected percutaneously, will break self tolerance to the autoantigen of the joint.

The influence of gastric exposure to antigen on the subsequent response to a specific challenge by the same or different route is well known. In the context of autoimmunity the demonstration of the concomitant development of local mucosal immunity and systemic tolerance following gastric exposure may be particularly important. It is suggested therefore that diet normally does not precipitate autoimmunity because local mucosal immunity excludes dietary antigens that cross-react with autoantigens but this exclusion mechanism may become ineffective with, for example, age or endocrine changes or as a result of infection or inbalanced diet. The intriguing prospect that systemic tolerance to an autoantigen could be re-established by per-gastric dosing requires attention.

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