INCREASED COLLAGEN METABOLISM IN GRANULOMATA INDUCED IN RATS DEFICIENT IN ENDOGENOUS PROSTAGLANDIN PRECURSORS

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ABSTRACT

Collagen metabolism was measured (in terms of various hydroxyproline (HP), DNA and protein ratios) in granulomata obtained after s.c. implantation of carrageenan-impregnated and untreated polyether sponges into normal and essential fatty acid deficient (EFAD) rats for 8 and 15 days. Collagen synthesis (HP/protein) in day 8 and 15 untreated granulomata was the same for both normal and EFAD rats, though collagen breakdown (total HP) appeared to be greater in EFAD granulomata on day 15. With carrageenan-impregnated sponges, collagen synthesis in EFAD granulomata was much greater than in normal granulomata on both day 8 and day 15. Ratios of protein and/or HP to DNA (probably indicative of cellular infiltration) were increased in EFAD rats with both sponge types, though this increase was less pronounced with carrageenan-impregnated sponges. It is suggested that endogenous prostaglandin (PG) production (markedly reduced during EFA deficiency) may exert a negative feedback effect on collagen metabolism during proliferative inflammation.

INTRODUCTION

Both the erosive and the proliferative changes occurring during rheumatoid arthritis are associated with alterations in collagen synthesis and metabolism, which vary according to the tissue[1-3]. Prostaglandins (PGs) of the E-series, which are produced in large amounts by rheumatoid synovia[4,5], inhibit bone collagen synthesis *in vitro*[6], but stimulate collagen synthesis in cultured chick embryo skin[7]. In vivo, PGE_2 and $PGF_{2\alpha}$, in pharmacological doses, inhibited foreign-body granuloma formation in rats[8] and indomethacin, an inhibitor of PG biosynthesis[9], stimulated ^{3}H-proline incorporation into collagen in similar granulomata[10]. No explanation was given, however, for this latter effect. Recently, we have shown that carrageenan-impregnated sponge-induced granuloma formation is increased in essential fatty acid deficient (EFAD) rats, particularly in relation to changes in body weight, when PGE production is very markedly reduced[11]. We suggested that the effect on granuloma formation might have involved changes in collagen synthesis. We now report that this effect of EFAD is indeed associated with increased collagen synthesis, suggesting that endogenous PGs may exert a negative-feedback effect on collagen synthesis in proliferative inflammatory tissue.

MATERIALS AND METHODS

Male Wistar rats (TNO Central Breeding Institute, Zeist, The Netherlands) weighing 230-270 g were used, caged in Makrolons containing Sol "Speedi-Dry" (Metallochemie, Ramondt, Holland), an absorbant bedding. EFA deficiency was produced as described previously[11] and the mean weights of EFAD and normal rats were carefully balanced at the start of the experiment. Polyether sponges were implanted, sub-cutaneously, into the backs of the rats and removed on day 8 or day 15 using the method described earlier[11]. In one group of EFAD and normal rats, untreated sponges were first soaked in 2% sodium carrageenan (Viscarin, Marine Colloids Inc., Springfield, N.J.) and then dried at 37°C for 24h before implantation. After removal, sponges and the adherent tissue capsules were freeze-dried and stored with desiccant before biochemical analysis. Subsequently, the samples were weighed and cut into small pieces for determination of their DNA[12], protein[13] and hydroxyproline[14] contents. These data were expressed as within-sponge ratios, as shown in Tables 1 and 2. In this way, no corrections needed to be made for differences in either body weight or granuloma weight, as had been necessary in previous investigations[11]

RESULTS

Granulomata induced with untreated sponges

The results of biochemical analysis of the freeze-dried granulomata obtained 8 and 15 days after implantation of untreated sponges are shown in Table 1. There was no difference between collagen synthesis in granulomata from normal rats and that in EFAD granulomata on either day 8 or 15, as indicated by the hydroxyproline/protein ratios. Despite this lack of any difference in collagen synthesis, the total amount of hydroxyproline in day 15 sponges from EFAD rats was significantly increased. Cellular infiltration into the EFAD sponges, as indicated by DNA levels, was decreased, when considered as total DNA on day 8 and also when related to hydroxyproline on days 8 and 15 and to protein on day 8.

Granulomata induced with carrageenan-impregnated sponges

The results of biochemical analysis of the freeze-dried granulomata obtained 8 and 15 days after implantation of carrageenan-impregnated sponges are shown in Table 2. (This experiment was carried out at the same time as that with untreated sponges). In contrast to untreated sponges, collagen synthesis (hydroxyproline/protein ratio) was markedly increased in carrageenan-induced granulomata from EFAD rats, when compared with controls, on both day 8 and day 15. This increased collagen synthesis was accompanied by an increase in the hydroxyproline/DNA ratio, on day 8, which indicates a decrease in cellular infiltration into the EFAD granulomata. The protein/DNA ratio was unaltered both on day 8 and day 15, indicating that the decreased cellular infiltration into these EFAD tissues was not as pronounced as with untreated sponges.

Table 1. Various parameters of granulomata induced in EFAD and normal rats by untreated sponges.

Parameter	day 8		day 15	
	Normal	EFAD	Normal	EFAD
Sponge wt before implantation (mg)	25.7	25.4	25.4	25.8
Protein (mg)[a]	1.77	1.54	1.63	1.81
Hydroxyproline (µg)[a]	91.1	88.8	93.1	106.8 ↑**
DNA (µg)[a]	63.1	40.2 ↓***	59.4	47.4
Hydroxyproline/Protein	51.5	57.7	57.0	59.1
Hydroxyproline/DNA	1.48	2.46↑*	1.56	2.29↑*
Protein/DNA	28.8	41.8 ↑*	27.3	38.6

All values are the means of 6 observations. [a] Corrected for sponge wt. before implantation. Significance of differences between normal and EFAD data was determined by the one-tailed Mann-Whitney U test: * $p<0.05$, ** $p<0.02$, *** $p<0.01$

Table 2. Various parameters of granulomata induced in EFAD and normal rats with carrageenan-impregnated sponges.

Parameter	day 8		day 15	
	Normal	EFAD	Normal	EFAD
Sponge wt before implantation (mg)	28.5	30.0	29.8	28.4
Protein (mg)[a]	2.23	1.71	2.07	1.97
Hydroxyproline (µg)[a]	110.0	123.1	97.9	125.3
DNA (µg)[a]	91.0	80.3	97.2	91.8
Hydroxyproline/Protein	49.0	73.0 ↑***	47.0	62.0 ↑***
Hydroxyproline/DNA	1.21	1.59↑*	0.98	1.34
Protein/DNA	24.8	21.9	21.1	21.5

All values are the means of 6 observations. [a] Corrected for sponge wt. before implantation. Significance of differences between normal and EFAD data was determined by the one-tailed Mann-Whitney U test: * $p<0.05$, *** $p<0.01$.

DISCUSSION

The results of the present investigation show that collagen synthesis in granulomata induced by untreated, polyether sponges is unaltered during EFA deficiency, whereas collagen synthesis in granulomata induced by carrageenan-impregnated sponges is markedly enhanced during EFA deficiency. Stassen and Kuyper[15] have shown that granulomata, induced in rats by the subcutaneous injection of purified carrageenan, are associated with marked stimulation of collagen synthesis. Thus, it appears that it was this carrageenan-stimulated synthesis of collagen, which was sensitive to EFAD in our study. Impregnation of sponges with carrageenan results in the production of large amounts of PGE[11,16], much greater, in fact, than with untreated sponges (Bonta, I.L., Parnham, M. J. and Adolfs, M.J.P., unpublished observations). Thus, since EFAD rats are deficient in the precursors of PGs, it seems likely that increased collagen synthesis in granulomata from these animals is related to a marked reduction in endogenous PGE. This was suggested as a probable explanation for the increased carrageenan-induced granuloma formation seen in EFAD rats[11], since treatment of normal rats with PGE_2 inhibits granuloma formation[8]. In this context, it is worth noting that an inhibitor of collagen synthesis has been obtained from homogenates of granulomata induced in normal rats, though its nature was not determined[17]. The removal of an inhibitory effect of endogenous PGE on collagen synthesis probably explains the stimulation of ^{3}H-proline incorporation into granulomata from indomethacin-treated rats[10]. Recently, Denko[18] has shown that ^{3}H-proline incorporation into several collagen-rich tissues is inhibited in EFAD rats. Furthermore, PGE stimulates collagen synthesis in cultured chick embryo skin[7]. However, these were normal, not inflamed tissues, It appears that, in inflamed tissues, which are associated with the production of large amounts of PGE, the reactivity of collagen synthesis to PGE is reversed.

Although collagen synthesis, in granulomata induced by untreated sponges, was unaltered by EFA deficiency, total hydroxyproline in these granulomata was significantly increased on day 15. This observation may well reflect advanced collagen breakdown in these EFAD granulomata, during which either free or dialyzable hydroxyproline is present in increased amounts (Shoshan, unpublished observations). Thus PGs, in the smaller amounts released by untreated sponges, may inhibit collagen breakdown, as well as inhibiting its synthesis in larger amounts. It is probable that the marked increase in collagen synthesis observed in EFAD granulomata, induced by carrageenan-impregnated sponges, may have obscured any concomitant changes in collagen breakdown.

The marked reduction in total DNA and the change in the ratio of DNA to hydroxyproline and protein, in EFAD granulomata induced by untreated sponges, probably reflects a general reduction in the available number of infiltrating inflammatory leucocytes, since EFAD rats are somewhat leucopenic[19]. This suggestion is further supported by the fact that the reduction was most noticeable during the early (day 8) phase of the inflammatory response when leucocyte infiltration is more pronounced. With carrageenan-impregnated sponges, the reduction in cellular infiltration, produced by EFA deficiency, was not as great as that with untreated sponges. Since carrageenan is a potent inducer of leucocyte infiltration[20], it is probable that its activity was not as markedly

affected by the slight EFAD leucopenia as was the inflammatory action of the untreated sponges. However, in both cases, the fibroblasts of the EFAD granulomata were still sufficiently stimulated to maintain collagen synthesis, despite the apparent reduction in inflammatory cell infiltration.

In conclusion, our results indicate that, when the production of large amounts of endogenous PGs is very markedly reduced, collagen synthesis, during the proliferative phase of chronic inflammation, is enhanced. Furthermore, it appears that, under conditions when endogenous PG production is relatively much less, further marked reduction enhances resolution of the granulomata. Thus, whereas relatively small amounts of endogenous PGs appear to inhibit collagen breakdown, increased amounts appear to inhibit collagen synthesis. This may reflect a negative-feedback effect of PGs, produced during inflammation, which could be of importance in the modulation of connective tissue proliferation in chronic joint disease. Such a mechanism is supported by the finding that chronic joint swelling in adjuvant arthritic rats was increased during EFA deficiency[21]. A negative-feedback effect of PGs is also supported by the recent finding that indomethacin increases the proliferation of cultured human synovial cells[22].

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