OCCURRENCE OF TYPE III COLLAGEN IN INFLAMED SYNOVIAL MEMBRANES : A COMPARISON BETWEEN NON RHEUMATOID, RHEUMATOID, AND NORMAL SYNOVIAL COLLAGENS.

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Summary

Comparison of normal, rheumatoid and inflamed synovia has shown that abnormal amounts of Type III collagen can be isolated from rheumatoid or inflamed synovia, either after digestion of the tissues with pepsin or as an abnormal polymeric form (F2PC) accounting for 10-15% of total polymeric collagen after EDTA treatment, but not from normal synovia. The amount of Type III collagen appears to be related to the degree of inflammation.

Differences in susceptibility to pepsin between the bulk of the polymeric collagen from rheumatoid and non rheumatoid synovia were also noted.

Introduction

Rheumatoid and normal synovial polymeric collagens were shown by Steven to differ in their susceptibility to pronase and to alkali treatment (1). In a study of nineteen patients with classical rheumatoid arthritis it was shown that a proportion of their polymeric collagen could be solubilized by pepsin in contrast to the fourteen age matched normal patients, and also to the thirteen non-rheumatoid inflamed synovia studied (2). The pepsin susceptibility is thus shown to be unrelated to the inflammatory situation occurring in rheumatoid arthritis. However, an abnormal although totally pepsin

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resistant collagen was found to be present in varying amounts in all the inflamed tissues, including rheumatoid arthritis, the amount seeming to relate only to the extent of the inflammation.

The present report describes studies carried out in order to more fully define the differences, and in particular to analyse the collagen species present in the synovial membrane.

Materials and Methods

Synovia were obtained from surgical synovectomy, post-mortem dissection and in a few cases by needle biopsy from the supra patella pouch. Histological examination was carried out to confirm the diagnosis by Dr.J.Ball. Polymeric collagen was prepared according to the EDTA method of Steven (3). The initial precipitate which could be collected on neutralisation of the acid suspended polymer will be referred to as FIPC. After 24h it was possible to harvest a second precipitate from the supernatants after removal of FIPC, and this is referred to as F2PC. Collagen molecules present in the synovial membrane were released from the tissue by pepsin and separated as described (4). Pepsin digestion of polymeric collagen was performed as previously described (5).

Electrophoresis on SDS-polyacrylamide gels (5%), both with and without mercaptoethanol reduction, was performed by the method of Furthmayr and Timpl (6). Amino acid analysis was performed as previously described (7). Carbohydrates were analysed after methanolysis on a Pye gas liquid chromatograph (8). Hydroxyproline analyses were carried out by a semi-automated modification of the method of Woessner (9).

Results and Discussion.

Differences in pepsin susceptibility between normal and rheumatoid FIPC were confirmed. Traumatic and infective synovitis were resistant to degradation by pepsin as were the normal controls. Pepsin digestion of the rheumatoid polymeric collagen gave rise to altered -chains on disc electrophoresis (Fig.1). Both the residue left after extraction of polymeric and the whole synovia were treated with pepsin to enable isolation of the collagen species. In the normal situation pepsin has very little effect on the intact synovial membrane, but in the rheumatoid and also in the inflamed synovia approximately 15% of the total collagen can be solubilised, the bulk of which precipitates in a manner similar to the Type III collagen

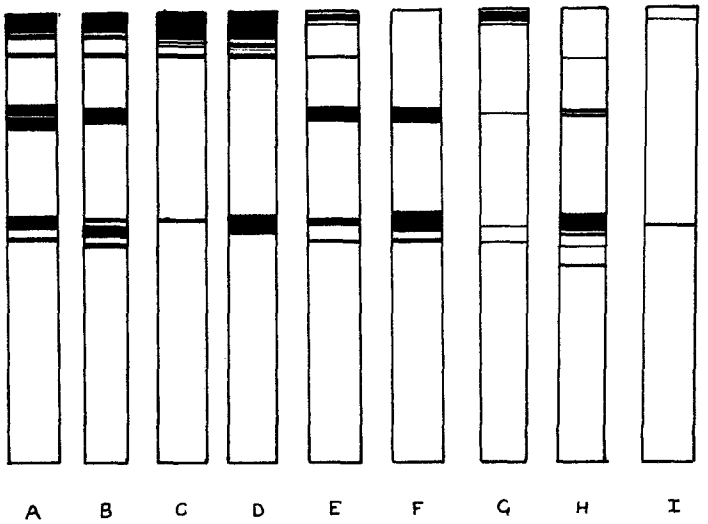

Figure I. SDS POLYACRYLAMIDE GEL PATTERNS :-

A) Type I collagen
B) Rheumatoid polymeric collagen digested with pepsin
C) Type III from rheumatoid synovium
D) Type III from rheumatoid synovium - with mercaptoethanol
E) Rheumatoid FIPC without mercaptoethanol
F) Rheumatoid FIPC with mercaptoethanol
G) Rheumatoid F2PC without mercaptoethanol
H) Rheumatoid F2PC with mercaptoethanol
I) Normal PC with mercaptoethanol

* No collagen entered the gel in the untreated normal PC.

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isolated from human skin (4). Disc electrophoresis of this material showed that it existed as high molecular weight components which on reduction with mercaptoethanol gave a single new band in the position of the α-chain (Fig.1). The material precipitating as Type I collagen gave both α_1 and α_2 bands on gel electrophoresis (Fig.1.).

Amino acid analysis of the isolated samples indicated certain similarities to the analyses previously reported for Type III collagen (4,10,11,12,13), notably the presence of ½ cystine, low values of valine and isoleucine and high values of histidine. (Table I). It was, however, a consistent finding that although hydroxyproline was higher in Type III collagen than in Type I the overall extent of proline

Table I amino acid analysis expressed as amino acid residues/1000 total amino acid residues. Tr signifies trace amounts.

	Type I	Type III	Normal FI	Rheumatoid F1	Rheumatoid F2
Hydroxyproline	87	104	92	96	88
Aspartic acid	52	45	56	46	54
Threonine	19	15	17	14	17
Serine	45	38	43	37	44
Glutamic acid	71	77	87	89	86
Proline	111	120	120	129	118
Glycine	337	340	339	336	334
Alanine	111	130	112	99	112
½ Cystine	-	1.2	Tr	Tr	1.2
Valine	20	12.5	11.1	12.1	12.6
Methionine	6.8	5.3	4.7	4.6	4.2
Isoleucine	12.4	6.5	5.7	5.6	4.7
Leucine	24	20	23	20.3	23
Tyrosine	4.7	2.7	3.7	3.8	4.7
Phenylalanine	10.7	11.7	12.0	9.6	11.3
Hydroxylysine	9.1	8.6	7.0	7.0	8.7
Lysine	23	19.2	21.4	17.0	23.0
Histidine	3.2	4.9	5.6	3.2	6.7
Arginine	47	42	39	36	46

hydroxylation was never as great as the previously published analyses.

Both rheumatoid and inflamed synovia gave rise to between 5 to 15% of the total polymeric collagen as F2PC (see material and methods section for definition). This form of polymeric collagen was

totally resistant to pepsin degradation, but could be partially depolymerised by heating in the sample buffer used for disc electrophoresis at 60° for 45 min. SDS gel electrophoresis showed that an α-chain component could be released from this polymer by treatment with mercaptoethanol, although traces of α-chain component were evidence before this treatment (Fig.1). Amino acid analysis confirmed the presence of ½ cystine in this fraction. Apart from a lower hydroxyproline value F2PC had a similar amino acid composition to that of the Type III collagen obtained by pepsin treatment (Table I).

Since normal synovia contained no F2PC and no extractable Type III collagen an examination of FIPC was carried out in order to determine if Type III collagen was present. Amino acid analysis was inconclusive (Table I), but it was possible to show by gel electrophoresis that treatment of this synovial collagen from normal tissue, with mercaptoethanol as previously described for F2PC, results in the release of a small amount of material which runs as α chains (Fig.1).

The presence in inflamed synovia (from whatever cause) of a novel form of polymeric collagen, which appears to consist largely of Type III collagen is of interest. The amount seems to relate to the degree of inflammation (2). Also of interest is the presence of detectable amounts of Type III collagen in the inflamed and rheumatoid synovia in contrast to the normal synovia.

The total resistance of F2PC, which seems to be a polymer of Type III collagen, to pepsin degradation poses questions as to the role of this cysteine containing protein in diseases where the presence of tissue proteases would be expected. A certain proportion of Type III collagen can be released from the tissue, however, by the action of pepsin and enables its isolation from rheumatoid and inflamed

synovia. Resistance to proteolytic degradation is presumably related to the structural organisation of the synovial membrane and to the nature and degree of cross-linking in its polymeric components. In this context it is of note that F2PC while sharing the resistance to peptide hydrolases can, unlike FIPC, be readily degraded by rheumatoid synovial collagenase (2).

The rheumatoid joint and the inflamed joint appear to be synthesising a type of collagen as a response to their inflammation, which in its soluble form is capable of resisting degradation and will thus allow for a controlled rate of turnover. Turnover may be faster in the rheumatoid than in the inflamed joint because of the sensitivity of F2PC to collagenase which would be present in abnormal amounts in the rheumatoid situation. This may account for the observation that there is always more F2PC in inflamed non-rheumatoid than in rheumatoid synovia (2). The nature of the abnormality of FIPC in rheumatoid synovia is still under investigation.

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