Type IX collagen is a potent inducer of PGE_2 and interleukin 1 production by human monocyte macrophages

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Type IX collagen and its collagenous fragments are potent stimulatory agents on human blood mononuclear cells for the production of prostaglandin E_2 and interleukin 1/mononuclear cell factor. Type IX collagen is 2–4-fold more potent that type I and II and 1α-, 2α- and 3α-collagens. This property may be important in the destructive process of cartilage in inflammatory diseases.

Collagen *Interleukin 1* *(Cartilage)*

1. INTRODUCTION

Type II collagen, containing 3 identical chains denoted α_1(II), is the major collagenous component of cartilage [1]. Recently, minor collagens, which represent about 5–10% of the total solubilized collagen cartilage have been described in different cartilaginous tissues, namely 1α-, 2α- and 3α-collagens [2], whose molecular organization is still unclear, and type IX collagen [3,4]. Type IX collagen molecules are disulfide-bonded heterotrimers of chain composition α_1(IX)α_2(IX)α_3(IX) and contain 3 helical domains separated by non-collagenous sequences [5] and glycosaminoglycan chains attached to the protein part [6]. Due to the presence of the non-collagenous domain, pepsin extraction of type IX collagen gives rise to several helical fragments characterized by their denaturation products on SDS-PAGE and termed X_1–X_7 in our laboratory [7]. These fragments, of different lengths, were tentatively placed in the native intact molecule in the model described in [8].

As yet there have been no functional studies on the biological role, if any, of type IX collagen. In previous studies we have found that not only collagen type II, but also types I and III, can stimulate the production of interleukin 1/mononuclear cell factor (IL-1/MCF) by monocytes [9]. This cytokine may play a significant role in the pathogenesis of tissue destruction in arthritic diseases through the stimulation of collagenase and prostaglandin E_2 (PGE_2) by synovial cells, chondrocytes and bone-derived cells [10]. Therefore, we have investigated whether 1α-, 2α-, 3α- and type IX collagens may also stimulate IL-1/MCF as well as PGE_2 production by human monocyte/macrophages.

2. MATERIALS AND METHODS

2.1. *Collagen preparation*

Type II, 1α-, 2α-, 3α- and type IX collagens were obtained from foetal calf epiphyseal cartilage by pepsin treatment and purified as described in [7,8].

Native X_4 was isolated from the other fragments of type IX collagen by molecular sieve chromatography on Biogel A-5m without denaturation. The column (2.5 × 100 cm) was eluted with 1 M $CaCl_2$–50 mM Tris (pH 7.5) at a flow rate of 17 ml/h.

Two fractions containing the denatured X_1–X_3 and X_4–X_7 fragments were obtained by molecular

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sieve chromatography of type IX collagen on Ultrogel AcA 22 under denaturing conditions. The column (2.5 × 100 cm) was eluted with 1 M $CaCl_2$, 50 mM Tris (pH 7.5) at a flow rate of 20 ml/h.

Type I and III collagens were extracted from human placenta by pepsin treatment and purified as in [11] and characterized by SDS-PAGE (fig.1), performed on 6.75 or 10% polyacrylamide gels as in [12].

2.2. *Culture plate preparations*

All the collagenous samples were dissolved in 0.1 M acetic acid at 1.0 mg/ml, at 4°C (native samples) or 40°C (denatured samples) for at least 24 h. Aliquots containing 100 μg collagen were placed in 16 mm diameter wells of tissue culture plates (Costar, Cambridge, MA) and allowed to dry at room temperature (native samples) or 40°C (denatured samples).

The plates were then placed under UV radiation for 1 h.

2.3. *Human peripheral blood mononuclear cells (PBMC) cultures*

Heparinized venous blood of healthy donors was layered on gradients of Ficoll-Hypaque (Ficoll-Paque, Pharmacia, Uppsala). The cell-containing interphase was aspirated, washed twice in Hanks' balanced salt solution (HBSS) and resuspended at 4 × 10^6/ml in RPMI 1640 (Gibco-Europe, Glasgow) supplemented with 2 mM L-glutamine, 10% heat-inactivated foetal calf serum (FCS, Gibco), 100 units/ml penicillin, 100 μg/ml streptomycin and 5 μg/ml polymixin B. Cell suspensions (500 μl containing 2 × 10^6 cells) were added to collagen-coated or uncoated 16 mm diameter wells. Cultures were incubated at 37°C, in a humidified atmosphere of 5% CO_2/95% air. After 72 h incubation, the cell-free supernatants were stored at −20°C and used for PGE_2 and IL-1/MCF determination on the basis of collagenase and PGE_2 stimulatory activity in human foreskin dermal fibroblasts (DF) or adherent rheumatoid synovial cells (ASC).

2.4. *Bioassay of IL-1/MCF*

Specimens of synovium from patients with rheumatoid arthritis obtained at the time of synovectomy and fibroblasts from human infant foreskin tissues were prepared and cultured as described [13]. For experiments, cells were cultured in flat-bottomed 96-multiwell plates (Costar) each containing 20000 cells/well per 200 μl Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% FCS, penicillin-streptomycin.

Monolayer cultures were exposed to diluted filtered (0.22 μm) PBMC culture supernatants. After 72 h, cell-free supernatants were stored at −20°C and used for PGE_2 and collagenase activity determination as in [13].

3. RESULTS AND DISCUSSION

3.1. *PGE_2 production by blood mononuclear cells exposed to collagens*

Blood mononuclear cells (BMC) exposed to different types of collagen and to concanavalin A (Con A) produced significant amounts of PGE_2 compared to BMC exposed to medium alone (table 1). In this experiment, as in 3 others (not shown), the level was highest when BMC were exposed to collagen type IX. However, human type I and bovine type II collagens were also active; the biological activities of these types of collagen have been reported previously [9].

We have examined the relative potency of the minor types of collagen from cartilage and some of

Table 1

Effect of different types of collagens on PGE_2 production by blood mononuclear cells

Collagen preparations	Blood mononuclear cells PGE_2 (ng/ml)
I (human)	21 ± 8
II (human)	4 ± 1
II (bovine)	11 ± 2
III (human)	4 ± 2
IX (bovine)	53 ± 17
1α-, 2α-, 3α- (bovine)	2 ± 1
Con A (25 μg/ml)	12 ± 1
No collagen	1 ± 1

Human blood mononuclear cells were added to collagen-coated wells at 4 × 10^6 cells/ml in RPMI 1690, 10% heat-inactivated FCS (see section 2). After 72 h, cell-free media were analysed for PGE_2 by a double-antibody radioimmunoassay [14] (antiserum from L. Levine, Brandeis University, MA). Values represent means ± SE (N = 4)

the native or denatured fragments of type IX collagen. In two separate experiments (table 2), type IX collagen and its fractions were clearly more potent than type II collagen. The molecules composed of 1α-, 2α- and 3α-chains were less active in both experiments. Fragment X_4 was less active in the denatured form.

The level of endotoxin measured by limulus assay (Microbiological Associates) in the medium after incubation with various preparations of collagens and especially with collagen type IX was less than 1.0 ng/ml. At this concentration no significant amounts of PGE_2 are produced by BMC.

3.2. *IL-1/MCF production by BMC exposed to collagens*

Media from BMC exposed to collagen were assayed for IL-1/MCF activity on dermal fibroblasts. As shown in table 3, type IX collagen was by far the most potent stimulus for IL-1/MCF measured by both PGE_2 and collagenase-stimulating activity. Except for type I collagen, the other collagens were less active in this experiment as well as in others (not shown). It has also been shown that collagen and collagen fragments can stimulate directly fibroblasts and synovial cells to produce PGE_2 and collagenase [14]. Medium originating from wells coated with collagens without BMC was not stimulatory for PGE_2 and collagenase by fibroblasts. This rules out the possibility of a carry-over of collagens or collagen fragments on the fibroblasts or synovial cells. In addition, endotoxin (LPS *Escherichia coli* 0111:B_4 Difco) even at concentrations as high as 1 μg/ml did not stimulate directly the fibroblasts or the synovial cells for PGE_2 and collagenase production.

As for PGE_2 production by BMC, we have also investigated the level of IL-1/MCF in supernatants from BMC exposed to different minor types of collagen and to native or denatured forms (table 4). In 3 different experiments and on 2 different target cells (i.e. synovial cells and fibroblasts), collagen type IX was more potent than the other collagens tested. The relative amount of IL-1/MCF induced

Table 2

Effect of different native and denatured preparations of type IX, II, 1α-, 2α-, 3α-collagens on PGE_2 production by blood mononuclear cells

Collagen preparations	Blood mononuclear cells PGE_2 (ng/ml)	
	Expt 1	Expt 2
Native		
IX: X_1–X_7	9.9 ± 2.1	160 ± 10
X_4	13.8 ± 0.5	ND
II	4.3 ± 0.5	73 ± 6
1α-, 2α-, 3α-	9.3 ± 1.1	38 ± 16
Denatured		
IX: X_1–X_7	24.8 ± 3.0	ND
X_4–X_7	ND	244 ± 14
X_1–X_3	ND	170 ± 54
X_4	3.8 ± 0.6	ND
II	3.1 ± 0.7	ND
1α-, 2α-, 3α-	2.8 ± 0.9	ND
Con A	22.0 ± 2.8	107 ± 30
No collagen	2.0 ± 0.2	2 ± 1

Human blood mononuclear cells were incubated as described in table 1. Expts 1 and 2 refer to two different healthy donors. Values represent means ± SE (N = 3); ND, not done

Table 3

Effect of different types of collagens on IL-1/MCF production by blood mononuclear cells measured by bioassay with dermal fibroblasts

Collagen preparation	Dermal fibroblasts	
	PGE_2 (ng/ml)	Collagenase (units/ml)
I (human)	7.5 ± 4.5	0.1 ± 0.1
II (human)	2.0 ± 0.1	0.5 ± 0.1
II (bovine)	2.0 ± 0.2	ND
III (human)	1.9 ± 0.3	0.3 ± 0.1
IX (bovine)	31.0 ± 4.0	1.7 ± 0.3
1α-, 2α-, 3α- (bovine)	2.5 ± 0.5	0.1 ± 0.1
Con A (25 μg/ml)	84 ± 10	1.3 ± 0.3
No collagen	0.3 ± 0.1	<0.1

Human blood mononuclear cells were incubated as described in table 1. After 72 h, cell-free media were diluted 1:40 in DMEM, 10% FCS and added to dermal fibroblasts. After 72 h, cell-free supernatants were assayed for PGE_2 level and collagenase activity after trypsin activation using [^{14}C]acetylated salt-soluble rat tail tendon. Values represent means ± SE (N = 3); ND, not done

Table 4

Effect of different native and denatured preparations of type IX, II, 1α-, 2α-, 3α-collagens on IL-1/MCF production by blood mononuclear cells measured by bioassay with synovial cells and dermal fibroblasts

Collagen preparations	Synovial cells				Dermal fibroblasts: PGE_2 (ng/ml)	
	PGE_2 (ng/ml)		Collagenase (units/ml)			
	Expt 2	Expt 3	Expt 2	Expt 3	Expt 2	Expt 3
Native						
IX: X_1–X_7	212 ± 27	135 ± 10	2.1	1.9	98 ± 6	31.4
X_4	94 ± 15	ND	1.5	ND	72 ± 6	ND
II	60 ± 7	96 ± 11	0.5	0.6	27 ± 1	13 ± 1
1α-, 2α-, 3α-	42 ± 28	30 ± 4	0.5	0.4	10 ± 8	3 ± 1
Denatured						
IX: X_1–X_7	205 ± 23	ND	2.6	ND	91 ± 12	ND
X_4–X_7	ND	211 ± 15	ND	1.9	ND	84 ± 7
X_1–X_3	ND	166 ± 8	ND	1.8	ND	73 ± 20
X_4	35 ± 4	ND	0.3	ND	17 ± 2	ND
II	29 ± 2	ND	0.1	ND	4 ± 2	ND
1α-, 2α-, 3α-	25 ± 3	ND	0.1	ND	2 ± 1	ND
No collagen	4 ± 1	9 ± 2	<0.1	<0.1	3 ± 1	9 ± 1

Human blood mononuclear cells were incubated as described in table 1. Cell-free supernatants were assayed on synovial cells or dermal fibroblasts as described in table 3. Expts 2 and 3 refer to two different healthy donors. Values represent means ± SE (N = 3); ND, not done

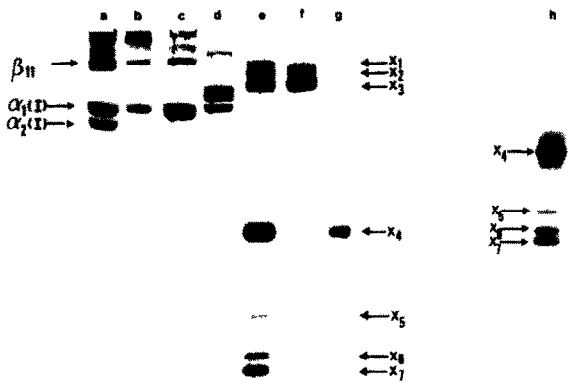

Fig.1. SDS-polyacrylamide gel electrophoresis (6.75% gel, a–g; 10% gel, h). Collagen types: (a) Type I, (b) type III, (c) type II, (d) 1α-, 2α-, 3α-, (e) type IX, (f) X_1–X_3 fraction, (g) X_4 fragment, (h) X_4–X_7 fraction.

by the other components follows the same pattern as for the PGE_2 released by the BMC. Fraction X_4 exhibited significant activity in the native form. However, when the denatured X_4–X_7 and X_1–X_3 fractions were tested high levels of IL-1/MCF were produced.

4. CONCLUSIONS

As previously reported collagens and collagen fragments are potent stimulatory agents of human blood mononuclear cells for the production of PGE_2 and IL-1/MCF [9]. Here, we have found that collagen type IX is 2–4-fold more potent than any other type of collagen tested. Fractions X_4, X_4–X_7 and X_1–X_3 were all active to various degrees. The levels obtained with these components cannot be explained only by endotoxin contamination since the level, as detected by the

limulus assay, was lower than that necessary to stimulate this system. However, we cannot exclude a synergism of the collagens with a very low level of endotoxin.

In a previous report we have found that collagens activate directly the monocyte-macrophages and that the lymphocytes are not necessarily required for this reaction [9]. This has also been found in our experiment using >95% purified monocyte preparations using adherence techniques (not shown).

The finding that type IX collagen and its fragments can stimulate PGE_2 and IL-1/MCF production by human monocyte-macrophages may be important in the destructive process of articular joint in inflammatory diseases. Monocytes may have receptors for collagen(s) [15] and further studies (i.e. binding) may be important for elucidating the mechanism of activation of macrophages in destructive arthropathies.

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REFERENCES

[1] Miller, E.J. and Matukas, V.J. (1969) Proc. Natl. Acad. Sci. USA 64, 1264–1268.
[2] Burgeson, R.E. and Hollister, D.W. (1979) Biochem. Biophys. Res. Commun. 87, 1124–1131.
[3] Shimokomaki, M., Duance, V.C. and Bailey, A.J. (1980) FEBS Lett. 121, 51–54.
[4] Ninomiya, Y., Showalter, A.M. and Olsen, B.R. (1984) in: The Role of Extracellular Matrix in Development, pp.255–275, A.R. Liss, New York.
[5] Van der Rest, M., Mayne, R., Ninomiya, Y., Seidah, N.G., Chretien, M. and Olsen, B.R. (1985) J. Biol. Chem. 260, 220–225.
[6] Bruckner, P., Vaughan, L. and Winterhalter, K.H. (1985) Proc. Natl. Acad. Sci. USA 82, 2608–2612.
[7] Ricard-Blum, S., Hartmann, D.J., Herbage, D., Payen-Meyran, C. and Ville, G. (1982) FEBS Lett. 146, 343–347.
[8] Ricard-Blum, S., Tiollier, J., Garrone, R. and Herbage, D. (1985) J. Cell. Biochem. 27, 347–358.
[9] Dayer, J.M., Trentham, D.E. and Krane, S.M. (1982) Collagen Rel. Res. 2, 523–540.
[10] Dayer, J.M. and Demczuk, S. (1984) Springer Semin. Immunopathol. 7, 387–413.
[11] Flandin, F., Herbage, D., Buffevant, C. and Bazin, S. (1981) Cell. Mol. Biol. 27, 677–685.
[12] Sykes, B.C. and Bailey, A.J. (1971) Biochem. Biophys. Res. Commun. 43, 340–345.
[13] Dayer, J.M., Zavadil-Grob, C., Uda, C. and Mack, B. (1984) Eur. J. Immunol. 14, 898–901.
[14] Biswas, C. and Dayer, J.M. (1979) Cell 18, 1035–1041.
[15] Hörmann, H. and Jelinic, V. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 87–94.