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Prostaglandin production in arthritis

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SUMMARY Inflammatory cell populations from synovial effusions or synovial villi in rheumatoid arthritis have been cultured *in vitro*. Prostaglandin productive capacity, measured by radioimmunoassay, showed the polymorphonuclear leucocyte rich populations from synovial effusions to be poor sources of PGE production whereas the synovial fragments produced substantial amounts of PGE activity. It is suggested that the macrophage is the major source of local prostaglandin formation both in gout and rheumatoid arthritis.

There is an interest in establishing the identity of mediators of the inflammatory response in rheumatoid arthritis and allied diseases in order both to aid the understanding of pathogenic processes in these diseases and to assist in discovery of novel anti-inflammatory compounds.

In 1971, Vane and his colleagues demonstrated that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) were potent inhibitors of prostaglandin synthesis (Ferreira *et al.*, 1971; Smith and Willis, 1971; Vane, 1971), so raising the likelihood that prostaglandins serve as inflammatory mediators in diseases in which such drugs are employed. Indeed elevated prostaglandin levels have been reported in synovial effusions from patients with rheumatoid arthritis (Levine, 1973; Higgs *et al.*, 1974; Patrono *et al.*, 1975; Swinson *et al.*, 1975) and from experimental animals in response to the injection of inflammatory stimuli (Blackham *et al.*, 1974; Glatt *et al.*, 1974). Additionally human rheumatoid synovial fragments have been shown to produce substantial amounts of prostaglandin during *in vitro* culture (Robinson *et al.*, 1973), but the cellular origin of prostaglandin-like material in such situations was not established. It has been reported that rabbit polymorphonuclear neutrophils generated prostaglandin-like activity in response to phagocytic stimuli (Higgs and Youlten, 1972; Higgs *et al.*, 1975), though others have found human peripheral blood neutrophils to be modest sources of prostaglandin production (Zurier and Sayadoff, 1975). In investigating the cellular origin of inflammatory prostaglandins in the guinea pig, the macrophage was shown to be a substantial source of prostaglandin production (Bray *et al.*, 1974), an observation particularly relevant to chronic inflammatory lesions where mononuclear cells are abundantly represented.

The present study was therefore undertaken to ascertain the prostaglandin productive capacity of inflammatory cell populations collected during arthrocentesis or arthroscopy from patients with clinically established rheumatoid arthritis or other inflammatory joint diseases.

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Methods

PATIENTS AND SAMPLE COLLECTION

Synovial tissue was obtained at knee joint arthroscopy in 8 patients, 6 of whom satisfied ARA criteria (Ropes *et al.*, 1958) for a diagnosis of classical or definite rheumatoid arthritis. One patient had gonococcal arthritis and 1 patient had systemic lupus erythematosus. Synovial fluid was obtained at knee joint arthrocentesis using a 19 G needle in 13 patients, 9 of whom satisfied ARA criteria for a diagnosis of classical or definite rheumatoid arthritis. Two patients had Reiter's disease, 1 psoriatic arthritis, and 1 osteoarthritis. At the time of the investigation all patients had active disease in the knee joint and arthroscopy or arthrocentesis were carried out for diagnostic or therapeutic purposes.

The patients were generally subject to treatment with NSAIDs and/or steroidal agents, although, at the time of investigation, no anti-inflammatory drugs were being prescribed for 3 of the patients (2 undergoing arthroscopy and 1 arthrocentesis).

CELL AND TISSUE CULTURE

Synovial effusions were filtered through gauze to remove mucin and fibrin clot, after which cells were separated from the synovial fluid by centrifugation at 400 *g* for 10 min and resuspended in Eagles Minimal Essential Medium (MEM) supplemented with 10% heat-decomplemented fetal calf serum (Flow Labs) to give a final cell concentration of $0{\cdot}2$–$1{\cdot}0 \times 10^6$ cells/ml. Arthroscopy fragments were washed in culture medium (MEM) and divided into smaller fragments approximately 1–2 mm^3. Cells or tissue fragments were cultured in an atmosphere of 5% carbon dioxide in air at 37°C for up to 24 hours. All synovial fragments were fixed and stained (haematoxylin and eosin—H & E) for evaluation of the cellular infiltrate.

ANIMAL STUDIES

Sterile inflammatory exudates were collected after intraperitoneal injection of starch (2% w/v in 0·9% saline) from guinea pigs and rabbits for neutrophil-rich populations at 4 h and for macrophage-rich populations at 72 hours. These cells were cultured in the same way as human cell populations.

PROSTAGLANDIN ASSAYS

Prostaglandin-like activity in culture supernatants was measured by direct radioimmunoassay using sheep antisera raised against PGE_2-bovine serum albumen (BSA) and $PGF_{2\alpha}$—BSA conjugates.

Anti-PGE antiserum cross-reacted 100% with PGE_2, 55% with PGE_1, 11% with 13,14-dihydro-PGE_2, 1·2% with 15-keto-PGE_2, 0·8% with 13,14-dihydro-15-keto-PGE_2, 1·5% with $PGF_{2\alpha}$, 0·6% with PGA_2 0·2% with PGB_2, and 0·3% with thromboxane B_2.

Anti-PGF antiserum cross-reacted 100% with $PGF_{2\alpha}$, 27% with $PGF_{1\alpha}$, 0·16% with 13,14-dihydro-15-keto-$PGF_{2\alpha}$, 0·21% with 15-keto-$PGF_{2\alpha}$, <0·13% with PGE_2, <0·013% with PGA_2, <0·016% with PGB_2, and 0·017% with thromboxane B_2.

Antiserum (0·1 ml of a dilution which bound 40-50% radio-labelled ligand) was equilibrated with tritium-labelled prostaglandin (5nCi in 0·1 ml, [5,6,8,11,12,14,15 ^{3}H]-PGE_2 160 Ci/mmole or [9-^{3}H]-$PGF_{2\alpha}$ 15 Ci/mmole, Radiochemical Centre, Amersham, UK) and unknown sample (0·1 ml diluted or undiluted) or standard solution of unlabelled prostaglandin (0·01–4·0 ng in 0·1 ml), in 0·1 mol/l phosphate buffer pH 7·4, at 4°C overnight.

Antibody-bound label was separated by the ammonium sulphate-precipitation method using bovine gamma globulin (2·5 mg) as carrier protein, and radioactivity determined using standard scintillation counting techniques. Prostaglandin activity of unknown samples was obtained by interpolation from standard curves, and expressed as PGE_2 or $PGF_{2\alpha}$ equivalents.

Results

Synovial effusions contained small quantities of PGE-like activity, with 5·8 ng PGE_2 equivalent/ml being the maximal concentration observed (Table 1).

Cells present in such effusions were predominantly neutrophil granulocytes. These cell populations have been cultured at concentrations of $0{\cdot}2$–$1{\cdot}0 \times 10^6$ cells/ml for periods of 24 hours. In 5 patients the observed levels of PGE-like activity in these cultures ranged from 0·44–1·92 ng PGE_2 equivalents/ml (Table 2). One patient with psoriatic arthritis was exceptional in producing considerably more PGE

Table 1 *Prostaglandin content of synovial effusions*

Case no.	*Diagnosis*	*Prescribed drug therapy*	*Cell count (in 10 ml effusion)*	*Prostaglandin concentration (ng PGE_2 equivalents/ml)*
1	RA	None	$6{\cdot}5 \times 10^6$	0·48
2	RA	DF118	Not done	2·60
3	RA	In, A	Not done	5·80
4	RA	B, A, N	$2{\cdot}3 \times 10^7$	0·63
5	RA	In, D	$1{\cdot}9 \times 10^8$	0·56
6	RA	In, D	$1{\cdot}0 \times 10^8$	0·47
7	RA	In, D	Not done	0·26
8	RA	P, D, A	$8{\cdot}6 \times 10^7$	0·15
9	RA	K	Not done	0·06
10	PA	In, K	$2{\cdot}9 \times 10^4$	1·64
11	OA	Ib	Not done	0·26
12	RS	None	$6{\cdot}7 \times 10^7$	0·07
13	RS	A	$2{\cdot}4 \times 10^8$	0·47

RA=rheumatoid arthritis. PA=psoriatic arthritis. OA=osteoarthritis. RS=Reiter's disease. A=aspirin. B=benorylate. N=naproxen. D=distalgesic (paracetamol + dextropropoxyphene hydrochloride). P=prednisone. Ib=ibuprofen. In=indomethacin. K=ketoprofen. DF118=dihydrocodeine tartrate.

Table 2 *Prostaglandin production by synovial effusion cells* in vitro

Case no.	*Diagnosis*	*Prescribed drug therapy*	*Cell culture concentration (cells/ml)*	*Post-culture viability (%)*	*Prostaglandin production (ng PGE_2/10^6 cells/24 h)*
3	RA	In, A	1×10^6	Not done	1·92
4	RA	B, A, N	1×10^6	84	1·06
5	RA	In, D	1×10^6	76	0·56
6	RA	In, D	1×10^6	91	0·44
10	PA	In, K	$0·2 \times 10^6$	Not done	46·0
13	RS	A	$0·8 \times 10^6$	84	0·70

Abbreviations as for Table 1.

activity than the rheumatoid patients, despite having the lowest cell count (Table 2).

Since rabbit peritoneal polymorphonuclear leucocytes have been reported to produce more PGE activity than human neutrophils following *in vitro* phagocytosis (Higgs and Youlten, 1972; Zurier and Sayadoff, 1975), inflammatory cell exudates from guinea pig and rabbit were collected and neutrophil-rich populations cultured in the presence and absence of phagocytic stimuli. Guinea pig neutrophils consistently produced comparatively small amounts of prostaglandins during short term culture (unstimulated cells produced 0·3–1·52 ng PGE_2 equivalents/10^6 cells/24 h; Table 3). Phagocytic stimuli included urate crystals, *B. pertussis*, and *E. coli* endotoxin, and the maximal production observed was 3·6 ng PGE_2 equivalents/10^6 cells/24 h in cultures containing urate crystals. Unstimulated rabbit cells produced 3·8–7·2 ng PGE_2 equivalents/10^6 cells/24 h (Table 3) although culture with *B. pertussis*, urate crystals or *E. coli* endotoxin caused little increase in PGE production. Guinea pig peritoneal macrophage-rich cell populations (60–80% macrophages) produced 11·6 ± SD 2·1 ng PGE_2 equivalents/10^6 cells/24 h (Gordon *et al.*, 1976). On the other hand, synovial fragments collected at synovectomy (1 case) or arthroscopy (7 patients) produced substantial concentrations of PGE activity and lesser concentrations of PGF activity during culture over a 24 hour period (Table 4). The prostaglandin productive capacity of these fragments was not clearly related to the character of the cellular infiltrate and did not depend upon the existence of necrosis, as the highest concentrations were observed in fragments in which necrosis was not evident. The time course of prostaglandin generation by such fragments is shown in Fig. 1, which demonstrates that prostaglandin formation represents *de novo* synthesis.

Figure 2 shows the dose-related inhibition of PGE and PGF formation by indomethacin. The concentrations of indomethacin required to achieve 50% inhibition of prostaglandin synthesis were

Table 3 *Prostaglandin production by guinea pig and rabbit peritoneal neutrophils during 24 hour cell culture. Effect of inflammatory stimuli*

Treatment	*Prostaglandin production (ng PGE_2 equivalent/10^6 cells/24 h) Mean (n=3)*	
	Rabbit	*Guinea pig*
No treatment	5·8	0·5
Urate crystals (low dose)	4·5	1·5
Urate crystals (high dose)	6·9	3·6
B. pertussis 1×10^8/ml	4·1	1·4
B. pertussis 2×10^8/ml	—	1·6
E. coli endotoxin		
10μg/ml	5·3	1·1
50μg/ml	7·2	1·1

Table 4 *Prostaglandin production of synovial fragments* in vitro

Case no.	*Diagnosis*	*Prescribed drug therapy*	*Prostaglandin production (ng/mg tissue/24 h)*		*Cellular infiltrate**			
			PGE_2	*$PGF_{2\alpha}$*	*Degree*	*Macrophages*	*Lymphocytes*	*Necrosis*
14	RA	None	52·7	9·1	3	1	3	0
15	RA	Pb, Ib	38·9	5·5	3	3	1	0
16	RA	A	39·7	Not done	3	2	3	2
17	RA	P, Pa	15·2	Not done	2	2	1	2
18	RA	D, M, Ab	10·8	Not done	3	1	3	0
19	RA	P, In, Dp	1·3	Not done	2	2	1	3
20	GA	Bp	53·8	1·6	2	2	1	0
21	SLE	Ib	11·6	Not done	2	2	1	2

RA=rheumatoid arthritis. GA=gonococcal arthritis. SLE=systemic lupus erythematosus. Pb=phenylbutazone. Pa=paracetamol. M=mogadon. D=distalgesic (paracetamol + dextropropoxyphene hydrochloride). Ab=ampicillin. Dp=penicillamine. Bp=benzyl penicillin. A=aspirin. P=prednisone. Ib=ibuprofen. *Cellular infiltrate was classified as: 1 = mild, 2 = moderate, and 3 = intense.

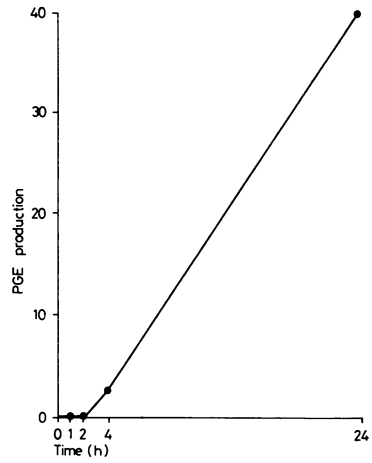

Fig. 1 *Time-course of prostaglandin production (ng PGE_2 equivalents/mg wet weight of tissue) by rheumatoid synovial fragments during* in vitro *culture. Each point represents the mean of 3 replicate samples (case 16).*

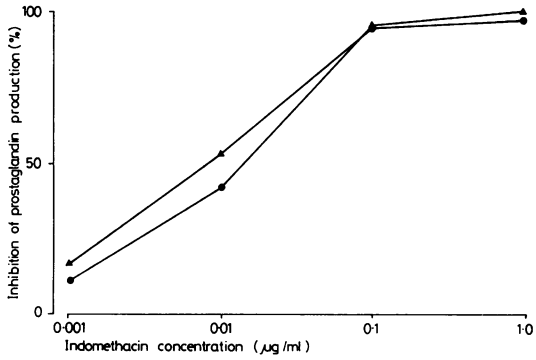

Fig. 2 *Inhibition by indomethacin of prostaglandin production by rheumatoid synovial fragments* in vitro. *Each point represents the mean of 2 replicate samples.* ▲—*$PGF_{2\alpha}$*, ●—*PGE_2.*

14·3 ng/ml and 8·4 ng/ml for PGE and PGF, respectively. Sufficient material was obtained from only one patient to attempt comparative drug evaluation. Untreated replicate samples (mean 7·5 mg, range 4·8–11·5 mg wet weight tissue, n = 10) showed substantial variation in PGE production (mean 80·3 ± SD 43·0 ng PGE_2 equivalent/ml) which was not markedly reduced by correction for sample tissue weight (mean 10·8 ± SD 5·4 ng PGE_2 equivalents/mg wet weight/24 h). Significant inhibition (> 50%) of PGE production was observed by indomethacin (0·001 μg/ml), dexamethasone (0·001 μg/ml), prednisone (0·001 μg/ml), hydrocortisone (0·1 μg/ml), ketoprofen (0·1 μg/ml), and naproxen (1·0 μg/ml), but phenylbutazone (1·0 μg/ml), feprazone (1·0 μg/ml), aspirin (1·0 μg/ml), and sodium salicylate (0·1 mg/ml) produced no detectable inhibition.

Discussion

It is difficult to obtain material from patients with active inflammatory joint disease who are not subject to treatment with anti-inflammatory drugs which are known to influence prostaglandin biosynthesis. In this study we have been able to include only 3 patients with no treatment; 1 with rheumatoid arthritis and 1 with Reiter's disease from whom synovial effusions were obtained whilst a third patient with rheumatoid arthritis was subjected to arthroscopy. In addition, 1 rheumatoid patient was receiving a simple analgesic and 1 case of gonococcal arthritis was being treated by antibiotic therapy and hence not subject to anti-inflammatory drug treatment.

The immunoreactive PGE content of synovial effusions from rheumatoid patients observed in this small study (Table 1) were generally consistent with those reported by Swinson *et al.* (1975), but were somewhat lower than those reported by Higgs *et al.* (1974), using bioassay techniques. Attempts to measure endogenous levels of primary prostaglandins in peripheral plasma have been complicated by the unavoidable activation of prostaglandin biosynthesis by cellular elements during sample collection (Samuelsson *et al.*, 1975). There is some evidence that a similar problem is encountered with prostaglandin determinations in synovial fluid (Swinson *et al.*, 1975). Higgs *et al.* (1974) reported comparatively high levels of both PGE_1- and PGE_2-like activities in synovial effusions from a larger series of untreated patients with rheumatoid arthritis, and it is notable that peripheral blood polymorphonuclear leucocytes released both PGE_1 and PGE_2 following phagocytosis *in vitro* (Zurier and Sayadoff, 1975). In contrast, rheumatoid synovial fragments produced PGE_2 almost exclusively, during *in vitro* culture (Kantrowitz *et al.*, 1975a). The significance of observed prostaglandin levels in synovial fluid is therefore uncertain, and determination of the prostaglandin synthesising capacity of rheumatoid cells during *in vitro* culture may provide a more reliable indication of events occurring *in vivo* than single synovial fluid samples. A comparable approach has been the determination of prostaglandin synthesising capacity of microsomal preparations derived from rheumatoid synovial tissue when incubated *in vitro* with exogenous arachidonic acid (Crook *et al.*, 1976).

The low prostaglandin synthesising capacity of synovial effusion cells (mainly polymorphonuclear leucocytes) during *in vitro* culture (Table 2) is consistent with the low level of prostaglandin production by human peripheral blood neturophils following *in vitro* phagocytosis (Zurier and Sayadoff, 1975), but contrasts with the prostaglandin productive capacity of synovial fragments (Table 4). Although rabbit peritoneal neutrophils have been reported to produce larger amounts of prostaglandins following *in vitro* phagocytosis (Higgs and Youlten, 1972) or during *in vitro* culture (Table 3) and prostaglandin synthetase has been characterised in lysates of rabbit neutrophils (Borgeat *et al.*, 1976) guinea pig peritoneal neutrophils were more closely in accordance with human cells. In the guinea pig, peritoneal macrophage-rich cell populations have been shown to possess much greater prostaglandin synthesising capacity than neutrophil-rich cell populations and it is noteworthy that their prostaglandin production may be further increased by products of lymphocyte activation (lymphokines) (Gordon *et al.*, 1976).

Lymphocytes which are abundant in rheumatoid synovial tissue might be considered as a potential source of prostaglandins. However, guinea pig lymph node derived lymphocytes produce little PG activity during *in vitro* culture (Gordon *et al.*, 1976) and PGE production by human spleen fragments has been shown not to be attributable to splenic lymphocytes (Ferraris and de Rubertis, 1974). Platelets also seem improbable as a source of E-type PG production by synovial fragments as prostaglandins are not major products of arachidonic acid metabolism by platelets (Hamberg *et al.*, 1975).

Synovial fragment were collected under direct vision as representing sites of active inflammatory response in the rheumatoid knee and such villous processes are comprised of a layer of synovial lining cells (several cells thick) overlying an infiltrate of mononuclear cells (Table 4) with few polymorphonuclear leucocytes evident. Although this study did not establish the cellular source of prostaglandins in these fragments, it seems more likely to be attributable to the infiltrating macrophages or phagocytic type A synovial lining cells which possess macrophage-like properties. This conclusion is supported by the observations that glass-adherent cells isolated from rheumatoid synovial fragments produced large amounts of PGE_2 (up to 1200 ng $PGE_2/10^6$ cells/24 h) during *in vitro* culture and that prostaglandin production occurred only during the period when the presence of macrophage markers could be detected (Dayer *et al.*, 1976).

An interesting extension of this conclusion is to question the role of polymorphonuclear leucocytes as a possible source of prostaglandins in gout; here we would suggest that the lining cells of the synovial membrane would provide the primary source of prostaglandins. This suggestion is strengthened by the observation that prostaglandin production precedes polymorphonuclear leucocyte infiltration in urate crystal-induced synovitis (Glatt *et al.*, 1974) while phagocytosis of monosodium urate crystals by macrophage-like synovial lining cells precedes polymorphonuclear neutrophil accumulation in experimental models of gout (Schumacher *et al.*, 1974). Furthermore, the development of spontaneous acute gout has been described in a patient with sever polymorphonuclear neutrophil depletion (Ortel and Newcombe, 1974).

Drug evaluation on synovial fragments was restricted by the availability of such material. Prostaglandin production by synovial fragments was inhibited by indomethacin (Fig. 2) at concentrations which approximate to free drug concentrations achieved in synovial fluid following therapeutic dosage (Emori *et al.*, 1973) and which are associated with symptomatic relief (analgesia) of rheumatoid arthritis (Brooks *et al.*, 1974). Studies in the guinea-pig have established the efficacy of both NSAIDs and steroidal drugs (Bray and Gordon, 1976) as inhibitors of PGE production by macrophages at therapeutic concentrations, whilst other studies of human synovial fragments have shown PGE production to be inhibited by steroids and NSAIDs (Kantrowitz *et al.*, 1975a; 1975b). Fragments of synovium proved less satisfactory for drug evaluation than dispersed cell populations. Thus phenylbutazone, feprazone, aspirin, and salicylate failed to inhibit PGE production at concentrations effective on dispersed cell populations (Bray and Gordon, 1976). Furthermore there is some indication that *in vivo* drug therapy diminished *in vitro* prostaglandin synthesising capacity of synovial fragments (Table 4).

Although aspirin irreversibly inhibits (by acetylation) the cyclo-oxygenase component of platelet prostaglandin synthetase (Roth *et al.*, 1975) and synovial microsomes derived from aspirin-treated rheumatoid patients have previously been reported to possess very little residual prostaglandin biosynthetic capacity (Crook *et al.*, 1976), synovial fragments from a patient receiving aspirin therapy (case 16, Table 4) showed substantial prostaglandin production during *in vitro* culture (see also Fig. 1). These results suggest that, in contrast to platelets, synovial tissue may rapidly recover (presumably by *de novo* enzyme synthesis) from inhibition by aspirin if therapy is discontinued and, hence, that platelets make little significant contribution to prostaglandin production by synovial fragments. It seems likely

that study of such tissue fragments may provide a more definitive test of theories concerning mediator involvement in rheumatoid arthritis than is afforded by the various animal models of arthritis, which in general have proved poorly effective in detecting novel anti-inflammatory drugs.

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