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COMMENTARY

ENDOGENOUS ANTI-INFLAMMATORY FACTORS

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In 1977 I reviewed the role of endogenous anti-inflammatory proteins in controlling inflammation [1]. It is now clear that proteins form only a part of complex endogenous defence systems against inflammatory diseases. In this commentary I shall discuss both protein and non-protein molecules involved in defence mechanisms against inflammation.

An important paper describing the anti-inflammatory properties of endotoxin [2] has thrown some doubt on work involving experiments where anti-inflammatory properties have been assigned to proteins separated from inflammatory exudates under non-sterile conditions [3]. Despite this apparent setback the concept of endogenous anti-inflammatory proteins has been firmly established with the discovery of the glucocorticoid induced anti-phospholipase A_2 proteins and the elucidation of the protective role of superoxide dismutases against inflammation. Both the phospholipase inhibitors and the superoxide dismutases show direct anti-inflammatory action against animal models of inflammation and we have a reasonable knowledge of their mode of action.

Anti-phospholipase A_2 inhibitory proteins

It has been known for some time the anti-inflammatory glucocorticoids inhibit prostaglandin synthesis by blocking the release of arachidonic acid from phospholipids [4] and that this effect was mediated through the synthesis of a 'second messenger' [5]. In 1980 Blackwell *et al.* [6] characterized this factor as an intracellular polypeptide (mol. wt 15,000) which they named as 'macrocortin', which was capable of inhibiting phospholipase A_2. In the same year Hirata and others [7] discovered a protein 'lipomodulin' [8] (mol. wt 40,000) with similar inhibitory properties against phospholipase A_2. More recently, two polypeptides (mol. wts 15,000 and 30,000) the 'renocortins' [9] with anti-phospholipase A_2 activity have been found in renal cells and a large anti-phospholipase A_2 protein (mol. wt 125,000) has been found to be released from rat peritoneal macrophages [10]. Apart from the common property of inhibiting phospholipase A_2 the production of these proteins was stimulated by exogenous glucocorticoids. Macrocortin synthesised by rat macrophages and lipomodulin by rabbit neutrophils were shown to be related since macrocortin showed cross-reactivity with antibodies against lipomodulin [11]. Lipomodulin is cleaved by proteinases to various active fragments including one of mol. wt 16,000 [7] and it has been suggested that macrocortin (mol. wt 15,000) is a fragment of lipomodulin [11] and that a high molecular weight precursor molecule (e.g. mol. wt 125,000) of both lipomodulin and macrocortin is synthesized in cells following stimulation by glucocorticoids. It is possible that a whole family of lower molecular weight anti-phospholipase A_2 proteins are released from this high molecular weight precursor molecule but this suggestion awaits definitive evidence [10]. Since anti-phospholipase A_2 polypeptides and proteins are released from macrophages and neutrophils, cells directly involved in the inflammatory process, they possibly act as modulators of inflammation. However, the occurrence of these steroid induced molecules in renomedullary cells indicates that anti-phospholipase A_2 proteins may have a more general regulatory physiological function [9]. A finding relevant to inflammatory disease was the detection of auto-antibodies against lipomodulin in the sera of patients with either systemic lupus erythematosus or rheumatoid arthritis [8], both chronic inflammatory diseases. Anti-phospholipase A_2 proteins induced by dexamethasone in rat peritoneal fluids inhibited carrageenan induced pleurisy in other rats showing that these proteins have a direct anti-inflammatory activity, at least against animal model systems [11]. In addition to the administration of exogenous steroids ACTH also promotes the release of these proteins *in vivo* [11]. Therefore, the release of anti-phospholipase A_2 proteins may represent an endogenous control mechanism against inflammation activated by the pituitary-adrenal axis in response to environmental stress situations [11, 12].

Superoxide dismutases

These molecules are found in all oxygen consuming organisms and in some aerotolerant and obligate anaerobes [13]. The enzymes are classified according to the type of metal found in their active centres. The copper zinc enzyme (Cu–Zn-SOD) is found in eukaryotic cytosols and the iron (Fe-SOD) or manganese (Mn-SOD) enzymes are found in prokaryotes. The manganese enzyme is also found in mitochondria. Superoxide dismutases catalyse the dismutation of thte superoxide anion:

$$2O_2^- + 2H^+ = H_2O_2 + O_2$$

with great rapidity. The first superoxide dismutase was isolated from bovine erythrocytes [14] and it was related to an anti-inflammatory protein Orgotein [15] isolated from bovine liver. A link was found between

the biochemical properties of superoxide dismutase and the anti-inflammatory properties of Orgotein when it was found that neutrophils generated superoxide anion during phagocytosis [16]. Superoxide is generated during the oxidation of NADPH by a plasma membrane oxidase [17] and is normally incorporated into the secondary lysosome during phagocytosis. In inflammation when reversed endocytosis ('frustrated phagocytosis') occurs [18] where the phagocyte fails to engulf the material attached to its surface receptor, superoxide anions and derived activated oxygen species escape into the extracellular compartment from lysosomes that fuse with the surface membrane.

Superoxide dismutases are not normally present in extracellular fluids in high concentration [19] and therefore the tissues and extracellular molecules are subject to damage from the various highly reactive oxygen species liberated. Extracellular superoxide dismutase (e.g. Orgotein) can protect the tissues by scavenging superoxide [20] and the suppression of the inflammatory response by exogenous superoxide dismutase has been demonstrated in models such as the reverse passive Arthus reaction in the rat [21], induced glomerulonephritis in the mouse [22] and carrageenan induced oedema in the rat footpad [22, 23]. Orgotein has also produced clinical improvements in arthritis in man [24]. In man Orgotein has low antigenicity [25] but it has one major disadvantage in that it has a plasma life of only approx. 4–6 min [26]. This means that large doses have to be used for it to be clinically effective. Attempts have been made to prolong the survival time of the drug *in vivo* by coupling it to polymers such as polyethylene glycol monomethyl ether (mol. wt 1900) Dextran T-70 (mol. wt 70,000) or Ficoll-70 (mol. wt 70,000) [26, 27]. These chemically modified superoxide dismutases have been shown to possess prolonged survival times *in vivo* and under experimental conditions to prolong the anti-inflammatory action of the enzyme [26, 28]. Superoxide anion has been implicated in at least three pathophysiological processes, namely oxygen toxicity, ischemia-induced tissue damage and inflammation [20]. Its primary physiological role in neutrophils and possibly in other phagocytic cells is in participating in the sequence of oxidative events associated with microbicidal killing [29]. It is unlikely that superoxide is directly responsible for all the microbicidal and pathophysiological events associated with its production. Superoxide is the conjugate base of the weak acid HO_2^- (pK 4.8) and in aqueous media shows a docility towards organic molecules. However, it is the starting material for the production of a whole family of active oxygen species [30]. The superoxide anion is produced from phagocytes stimulated by a variety of agents such as zymosan, plant lectins, phorbal myristic acetate (PMA) [31]. After a short lag phase a significant amount of superoxide is transformed by complex and as yet not fully understood secondary reactions to the hydroxyl radical ($OH\cdot$) 'hypochlorous acid' (HOCl) hydrogen peroxide (H_2O_2), singlet oxygen ($O_2\cdot$) peroxy radicals ($HO_2\cdot$) and hydroperoxides (ROOH) [32]. Microbicidal activity is associated with the hydroxyl radical, hypochlorous acid and to a lesser extent hydrogen peroxide [29]. At least three activated oxygen species have been associated with inflammation. Superoxide anion itself may play a direct role by producing a powerful chemotactic factor in plasma [23]. The factor has not yet been identified but it can be extracted by chloroform from serum albumin after treatment of the serum with superoxide [23]. This factor is possibly related to other chemotactic oxidized lipid products but is bound to albumin for activity. Superoxide dismutase but not catalase can prevent the formation of this radical so it appears that the superoxide anion is responsible for the formation of the chemotactic factor rather than hydrogen peroxide or radicals derived from hydrogen peroxide [23]. The inhibition of the formation of this chemotactic factor may represent an important clinical effect of Orgotein although a possible successful treatment will demand the development of clinically acceptable Orgotein derivatives with prolonged survival times *in vivo*. Superoxide anion is also believed to be responsible, at least in part, for the depolymerization of hyaluronic acid, an event often found in the synovial fluids of patients with inflammatory arthritic disease. Experiments *in vitro* have shown that this depolymerization can be prevented by superoxide dismutase, catalase, mannitol and histidine [19]. Catalase will remove hydrogen peroxide which is produced by the dismutation of superoxide anion, mannitol is a scavenger for the hydroxyl radical [33] and histidine for singlet oxygen [34]. Therefore several active oxygen species appear to have the property of depolymerizing hyaluronic acid. Iron is believed to play a proinflammatory role in arthritis by catalysing the activation and production of oxygen species. Iron chelating compounds such as desferrioxamine have shown anti-inflammatory action in animal models [35]. In arthritis iron accumulates in the synovial membrane [36]. The pro-inflammatory role of iron is believed to be by the interaction of the metal ion with the Haber–Weiss reaction [37]:

$$O_2^- + H_2O_2 = O_2 + OH^- + OH^\cdot.$$

This non-catalysed reaction is probably too slow to be of physiological significance but is catalysed by transition metal ions (e.g. Fe^{2+}):

$$Fe^{2+} + H_2O_2 = OH^\cdot + Fe^{3+} + OH^-$$

$$Fe^{3+}\text{-complex} + O_2^- = Fe^{2+}\text{-complex} + O_2. \text{ [35]}$$

The second reaction regenerates the ferrous form of iron. The toxic product of the iron catalysed Haber–Weiss reaction is the hydroxyl radical [38] which is very reactive against tissues. Polyunsaturated acids are susceptable to free radical attack leading to the formation of peroxy and alkoxy free radicals capable of initiating chain reactions and the formation of lipid peroxides [39]. Since polyunsaturated acids are concentrated in membranes lipid peroxidations will result in the widespread destruction of cellular and organelle membranes, the release of lysosomal enzymes, chemotactic factors and altered tissue components which perpetuate inflammatory disease. The superoxide dismutases represent the first line of defence against excess superoxide formation in cells and catalase and glutathione peroxidase form a

second line of defence by removing hydrogen peroxide and other activated oxygen species.

A possible function of the superoxide dismutase–catalase system in arthritis apparently not discussed in the literature may be in the reoxygenation of the tissues in inflammation. It is well known that in active inflammatory arthritis the oxygen levels in joint tissues and fluids fall to low levels [40]. This is probably due to the stimulated neutrophils, monocytes and macrophages (e.g. synovial A cells) metabolizing abnormally high amounts of oxygen. The superoxide dismutase–catalase system will regenerate some of this oxygen and make it available to the tissues. In a recent study in children the superoxide dismutase content of cells from rheumatoid arthritis patients were lower than in healthy controls [41] and Orgotein is clinically effective when administered by intra-articular injection [42].

Anti-proteinases

Anti-proteinases are distributed in cells and fluids in all living systems. They act as homostatic regulators of proteolytic action in extracellular fluids and have regulatory functions within cells. This latter function will be discussed later. Eight or nine anti-proteinases are present in human plasma [43], the most important being alpha-1-proteinase inhibitor (mol. wt 52,000 290 mg%), alpha-1-chymotrypsin inhibitor (mol. wt 69,000 49 mg%J), inter-alpha-trypsin inhibitor (mol. wt 160,000 50 mg%), alpha-2-anti-plasmin (mol. wt 70,000 7 mg%), anti-thrombin-3 (mol. wt 65,000 24 mg%), C-1-inactivator (mol. wt 70,000 24 mg%) and alpha-2-macroglobulin (mol. wt 720,000 260 mg%) [44].

Inter-alpha-trypsin inhibitor may dissociate both *in vitro* and *in vivo* to form stable acidic protein anti-proteinases which can pass through the kidney and appear in the urine as 'mirgin' [45]. Alpha-2-macroglobulin is really a proteinase 'trap' since it will irreversibly and non-specifically bind with all proteinases. The alpha-2-macroglobulin-bound proteinases retain their proteolytic activity towards low molecular weight substrates, showing that their active sites are still free. However, the alpha-2-macroglobulin-bound proteinases are unable to attack high molecular substrates because 'masking' by the large protein prevents contact between the enzyme and its substrate [46]. This may not be relevant under physiological conditions since the clearance time for alpha-2-macroglobulin-proteinase complexes in man is 10 min [47]. Under *in vitro* conditions some proteinases (e.g. elastase) may release themselves from the complex by proteolytic action and resume activity against high molecular substrates [48]. Alpha-1-proteinase inhibitor and alpha-1-chymotrypsin are acute phase reactants in man [49]. The accepted role for plasma anti-proteinases is in inactivating and removing extracellular proteinases from the circulation. C-1-inhibitor and alpha-2-macroglobulin are the major inhibitors of plasma kallikrein. Alpha-1-proteinase inhibitor and alpha-2-macroglobulin remove granulocytic derived collagen and elastase [45], alpha-2-antiplasmin reacts rapidly with plasmin and anti-thrombin-3 is the major thrombin directed anti-proteinase but may require heparin for its activity *in vivo* [50]. Inter-alpha-trypsin inhibitor inactivates trypsin-like enzymes. Alpha-1-proteinase inhibitor and alpha-2-macroglobulin are the two major contributors to the anti-proteinase activity of plasma.

Alpha-1-proteinase inhibitor can react with active oxygen species. The ability of alpha-1-antiproteinase to bind and inactivate elastase was lost in the presence of active oxygen species generated either by neutrophils stimulated by PMA or by myeloperoxidase in the presence of hydrogen peroxide and chloride ions [51]. The inactivation from active oxygen species from both sources was very rapid but was competitively inhibited by methionine and blocked by catalase. Superoxide dismutase had no protective effect. From these observations it appears that the inactivating factor was hypochlorous acid or other active species generated by a myeloperoxidase–hydrogen peroxide–halide system. Other experiments have shown that methionine residues in alpha-1-proteinase inhibitor are sensitive to oxidation by active oxygen species [52]. The properties of alpha-1-proteinase inhibitor was examined in broncho-alveolar lavage fluids from patients with acute or chronic inflammatory pulmonary disease. It was found that the alpha-1-proteinase inhibitor from this source failed to bind ^{125}I-trypsin and did not inactivate porcine pancreatic elastase. On further investigation it was found that active oxygen species, probably from stimulated neutrophils or macrophages, had oxidized methionyl residues in the anti-proteinase to the corresponding sulphoxide. The presence of methionyl sulphoxide residues in the anti-proteinase resulted in its degradation by porcine elastase to a smaller (mol. wt 47,000) inactive fragment. It was suggested that alpha-1-proteinase inhibitor was acting as an oxidant trap in the lavage fluids [52]. However, the inactivation of alpha-1-proteinase inhibitor may increase superoxide production from neutrophils and macrophages [53]. Exogenous serine proteinases have been shown to stimulate superoxide production from cells stimulated with either cytochalasin B or concanavalin A [54]. Under physiological conditions it is more likely that serine proteinases bound to the surface membrane of the phagocyte are involved since in experiments where human basophils were stimulated with anti-IgE it was found that superoxide generation was inhibited by treating the cells with serine proteinase inhibitors [55]. Plasma anti-proteinases may also affect lymphocyte function [56]. Serine proteinases such as trypsin, chymotrypsin, elastase, thrombin and PMN proteinases were found to stimulate DNA synthesis in mouse [57] or hamster [58] lymphocytes stimulated either by mitogens or allogenic responses and the treatment of lymphocytes with serine proteinase inhibitors such as alpha-1-proteinase inhibitor, alpha-2-macroglobulin, aprotinin or Soy bean tryptic inhibitor depress DNA synthesis [59]. Although alpha-2-macroglobulin and alpha-1-proteinase inhibitor are synthesized in the liver they are also synthesized in monocytes [60] and as these proteins act as a regulatory molecules in monocyte–lymphocyte interactions their inactivation may have important pathophysiological consequences. Although the body has an adequate supply of anti-proteinases deficiencies may occur in local situations in joints [61]. Such a situation may arise in the cartilage–pannus junction in arthritis

where the loss of cartilage is most pronounced. Intense local action by stimulated phagocytes in a close environment may well lead to a build up of activated oxygen species and proteolytic enzymes. The local inactivation of serine proteinases may well further the production of activated oxygen species which together with liberated proteinases will attack adjacent tissues. Although alpha-2-macroglobulin is not inactivated by oxygen species inactive forms of this anti-proteinase are found in arthritic joints and in some cases no active anti-proteinase was detected in synovial fluids [61]. In a close environment alpha-2-macroglobulin would have to diffuse through tissues and its high molecular weight would slow its rate of diffusion.

We have found that under aseptic conditions alpha-1-proteinase inhibitor does not inhibit carrageenan induced oedema in the rat. However, when we supplemented the rat's endogenous alpha-1-proteinase inhibitor supply with daily injections (sc) of alpha-1-proteinase inhibitor (100 mg/kg) we found a significant anti-inflammatory effect against adjuvant induced arthritis in the rat (unpublished results). This result is consistent with an anti-arthritic role for alpha-1-proteinase inhibitor rather than an anti-inflammatory one. Alpha-1-proteinase inhibitor levels rise in blood and synovial fluid in rheumatoid patients. It is an established acute phase protein in man. It is not an acute phase protein in the rat but the plasma levels rise in response to glucocorticoid treatment [62].

Eicosanoids and related factors

The pro-inflammatory properties of the prostaglandin E series (PGEs) was recognized early in the last decade [63]. It became clear throughout the decade that PGEs acted as powerful vasodilators and that by enhancing, although not directly producing, the actions of histamine and bradykinin in producing pain and oedema [64, 65] they were major contributors in producing three of the cardinal signs of inflammation. Other products of the cycloxygenase pathway were implicated in inflammatory processes such as prostacyclin (PGI_2) with vasodilatory and hyperalgesic properties [74] and thromboxane A_2 (TXA_2) which promotes platelet aggregation [65–67].

The role of prostaglandins and related products has expanded considerably over the last decade. Small oxidant radicals released by prostaglandin hydroperoxidase in the conversion of PGG_2 to PGH_2 may be responsible for some of the cellular damage that accompanies chronic inflammation [68] as well as inactivating certain enzymes in the cycloxygenase pathway in the absence of anti-oxidants [69]. Another important finding was that products of the 5-lipoxygenase pathway—the leukotrienes—were associated with slow reacting substances of anaphylaxis (SRA-A) and that Leukotriene B_4 (LTB_4) was a very powerful chemotactic attractant to neutrophils [70]. Recent work has considerably expanded the list of arachidonic acid derivatives with roles or possible roles in inflammation. Thromboxane A_2 LTD_4 and LTC_4 have been found to possess vasoconstrictor activity [71] and LTB_4 LTC_4 and LTD_4 also have permeability increasing activity [71]. The roles of these inflammatory mediators varies with species and the nature of the inflammatory stimulus. Different cells produce different derivatives and some products antagonize the production of others. Prostacyclin is a potent antagonist of the platelet aggregating properties of TXA_2 [72] and 15-HPETE, a product of the 15-lipoxygenase pathway, inhibits prostacyclin synthetase and therefore the production of prostacyclin [73]. It is beyond the scope of this commentary to discuss in detail pro-inflammatory events associated with the products of the arachidonic acid cascade but the subject has been extensively reviewed [67, 72, 74].

The physiological environment has significant effects on both PG synthesis and their reactions *in vivo*. A convenient *in vivo* model system for studying the effects of conversion of arachidonic acid (AA) to active derivatives such as PGI_2 or TXA_2 is the fall in blood pressure in either the rabbit or the rat following the i.v. injection of AA [75]. Various environmental factors have been shown to affect blood pressure in this model. Heparinisation of rabbits potentiates binding between AA and plasma proteins making more AA available for PG synthesis [76]. The fact that PG synthesis was involved was shown when the hypotensive effect of amino acids was abolished by treatment with indomethacin. Heparin itself does not have a direct action on PG synthesis by bull seminal vesicles [77] thereby showing that its effect on rabbit blood pressure is indirect. Heparin does not normally occur free in blood but can be released quickly from mast cells by anaphylactic shock.

The acute-phase protein haptoglobin, either by itself or in combination with haemoglobin, inhibited the synthesis of PGs [78], possibly by reducing the endoperoxides produced by the action of cycloxygenase on AA. Haptoglobin has direct anti-inflammatory activity against carrageenan induced paw oedema in the rat [79]. Other endogenous substances modify AA metabolism in the rabbit. Uric acid lowered the AA_{50} in producing hypotension [80, 81]. Uric acid is a potent hydroxyl free radical scavenger and the removal of this active radical may have prolonged the life of cycloxygenase [81]. Other purines (adenine, hypoxanthine and xanthine) [82] cysteine, tryptophan and glutathione [83] also enhance the hypotensive effect of AA. Singlet oxygen quenchers such as lipoic acid inhibit PG synthesis *in vivo*.

The relationship of oxygen metabolites to prostaglandin synthesis has been discussed by Deby [84, 85]. Low concentrations of hydroperoxides are required for cycloxygenase action [86] and low concentrations of reducing agents stimulate cycloxygenase action [86]. However, high concentrations of hydroperoxides can deactivate enzymes of the prostaglandin biosynthetic pathway and compounds with reducing groups can stimulate AA metabolism by scavenging damaging oxidants [87]. Therefore at the cell level the concentrations of reducing agents present will act as control mechanisms on PG synthesis either by stimulating or inhibiting AA metabolism according to the amount of reducing agent present.

At the clinical level the finding that certain non-steroidal anti-inflammatory drugs such as aspirin and

indomethacin were potent cycloxygenase inhibitors offered chemists the opportunity to produce new powerful drugs. [88, 89]. Unfortunately, the clinical efficacy of cycloxygenase inhibitors has never matched promising laboratory results. No clear evidence has yet emerged to show that NSAID cycloxygenase inhibitors have shown substantial clinical benefits by retarding the underlying disease process in arthritis. However, by relieving inflammatory symptoms such as pain and swelling they have undoubtedly brought relief to patients.

Although some NSAID cycloxygenase inhibitors, e.g. indomethacin do show some lipoxygenase inhibiting ability *in vitro* [90] there is no convincing evidence that they have a potent lipoxygenase inhibiting effect *in vivo*. Consequently, efforts are being made to produce powerful lipoxygenase inhibitors which will suppress *in vivo* the chemotactic actions of the leukotrienes without showing the adverse effects of the glucocorticoids. Unfortunately, one such drug benoxaprofen which is a potent lipoxygenase inhibitor, with some cycloxygenase inhibiting capacity as well, proved to have toxic side-effects in patients.

In general the inhibition of cycloxygenase can occur through three types of reaction [91] reversible competition, irreversible competition and reversible non-competitive competition for active sites on the enzyme. Fatty acids closely related to the substrate with a competitive affinity for the enzyme provide examples of the first type. These fatty acids are not converted to oxygenated products. An example of this type of action is provided by the anti-inflammatory drug Ibuprofen [92]. The action of aspirin provides an example of irreversible competition since it covalently acetylates a lysine residue in the active site of the enzyme [93]. Reversible non-competitive competition is shown by a range of both endogenous or exogenous compounds with anti-oxidant or radical trapping properties. Cycloxygenase activity is sustained by the continual presence of lipid peroxides that induce a free radical reaction. This is blocked by free radical scavengers or anti-oxidants when present in sufficient quantity and this is probably the basis of the action of paracetamol [94]. There are no lipoxygenase inhibitors which do not also have some inhibitory action against cycloxygenase. This makes it difficult to study their action on the production of lipoxygenase products. Attempts are being made to block lipoxygenase action by synthesizing acetylinic analogues of AA which will compete with natural substrates for active sites on the enzyme. An effective lipoxygenase inhibitor (which chemically inhibits the synthesis of PGs, hydroxy acids and leukotrienes) is BW 755C (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline) (a pyrazoline derivative of phenidone) which acts by inhibiting the peroxidation of AA. This substance is proving to be a useful biochemical tool in studying eicosanoid synthesis [95].

Although some NSAIDs inhibit individual enzymes in eicosanoid synthesis some NSAIDs such as indomethacin, sodium meclofenamate and sodium flufenamate inhibit phospholipase A_2 action, though by a different action than that of the glucocorticoids. Phospholipase A_2 in rabbit alveolar macrophages, rabbit neutrophils and in human platelets require Ca^{2+} for activation, possibly *via* calmodulin which binds to phospholipase A_2 [96]. Some drugs, such as the three above, interfere with the uptake of Ca_{2+} by the enzyme [97].

Although the role of PGs and certain analogues as pro-inflammatory mediators is well established, evidence is accumulating that they have anti-inflammatory and regulatory roles in chronic inflammation. The anti-arthritic properties of PGs became apparent when it was found that PGEs were able to inhibit pannus formation in the adjuvant arthritic rat [98] and cotton wool induced granuloma in the rat [99]. The anti-arthritic properties of the PGEs are believed to be linked with their ability to raise cyclic AMP levels in many cell types [100]. The raising of intracellular cyclic AMP levels tends to inhibit functions associated with inflammation such as a reduction in lymphocyte stimulation, cell mediated cytotoxicity, decreases in antibody and lymphokine production and an inhibition of the release of inflammatory mediators such as histamine and lysosomal factors including the destructive lysosomal hydrolases [100]. Cyclic AMP levels change during the development of chronic inflammation. When carrageenan induced granuloma in the rat was used as a model of chronic inflammation it was found that over a 7 day period as granuloma developed the cyclic AMP levels in macrophages isolated from the diseased tissues fell [101]. This fall in cyclic AMP was related to the PGE_2 content of the macrophages. Early stage macrophages produced adequate PGE_2 to sustain cyclic AMP levels. Later stage macrophages lacked endogenous PGE_2 and therefore produced less cyclic AMP. The lower cyclic AMP levels *in vivo* were associated with the growth of granulation tissue with PGE levels a controlling factor. Drugs that raise the cyclic AMP levels either by stimulating its synthesis *via* adenyl cyclase (e.g. PGEs or beta-adrenoreceptor agonists such as salbutamol or isoprenaline) or by preventing its breakdown by inhibiting phosphodiesterase (e.g. theophylline) show synergistic anti-inflammatory action against animal models of inflammation. Combinations described in the literature include theophylline and PGE_1 [102] and salbutamol and aminophylline [103]. Cyclic AMP also stimulates ornithine decarboxylase (ODC) activity and two oligoamines produced by ODC, spermidine and putrescine, show anti-inflammatory properties [104]. Putrescine is anti-inflammatory against carageenan induced foot oedema in the rat (an acute inflammatory model) and adjuvant induced arthritis in the rat (a chronic inflammatory model). Isoprenaline shows anti-inflammatory activity against carrageenan induced inflammation in the rat [105]. This effect by isoprenaline is reduced but not abolished by treating the rats with difluoromethylornithine (an ODC inhibitor) before injecting the carrageenan which suggests that cyclic AMP and oligoamines have separate anti-inflammatory actions [105]. Putrescine and spermidine are efficient scavengers of active oxygen species and have been shown to protect lysosomes from superoxide induced lysis *in vitro* [106] but whether this action is significant *in vivo* has yet to be proven. It is well established that cycloxygenase-derived products are increased in inflammation [107]

and PGI_2 (as its stable metabolite 6-keto-PGF_{1a}) and other eicosanoids such as PGE_2 and TXA_2 have been identified in inflammatory exudates [108, 109]. The sources of these products in arthritic joints is uncertain although it is known that neutrophils are relatively poor producers of PGEs and the release of PGEs from neutrophils is not sustained [110]. Monocytes are better producers of cycloxygenase products than neutrophils and stimulated macrophages release large amounts of PGE_2, PGI_2 and TBX_2 over a sustained period [111]. In the arthritic joint the synovium becomes increasingly cellular and 'A cells' develop which have macrophage-like properties and these cells and interstitial cells contribute to the production of cycloxygenase products in the disease process [112, 113]. The neutrophil is an important cell in active rheumatoid arthritis. About 90% of the cells found in the synovial fluids of patients with active disease are neutrophils [114]. It is characteristic of neutrophils that they degranulate and invade tissues when they encounter cartilage with entrapped immune complexes [115]. Stimulated neutrophils release lysosomal enzymes, reactive oxygen species and lipid derived products such as PGs, LTs and platelet activating factor [116]. The neutrophil can be stimulated by a wide range of secretogogues including immuno-complexes, lectins such as concanavalin A, PMA and lipid and water soluble ionophores such as the two fungal ionophores A23187 and ionomycin and by arachidonic acid or LTD_4. Different receptors exist for different secretogogues, e.g. Ig coated particles bind to Fc receptors and C_{3b}-opsonsized particles bind to C receptors [117]. The secretogogues 'trigger' the mobilization of Ca^{2+} leading to a rise in intracellular Ca^{2+} followed by rearrangements in microfilament and microtuble structures and the activation and release of neutrophil products [for details of secretogogue actions see 118]. Two neutrophil products, phosphatydic acid and LTB_4 may act as calcium ionophores [116]. Prostaglandins and in particular PGE, PGE_2 and PGI_2 inhibit neutrophil functions by reducing chemotaxis, aggregation, lysosomal enzyme release, the formation and release of superoxide and other oxygen species and also lipid derived products [119]. The common feature of PG modulating action appears to be that PGs link to surface receptors and raise intracellular cyclic AMP levels [117]. Other agents such as beta-adrenergic catecholamines and histamine also bind to neutrophil receptors and raise cyclic AMP levels [120]. A common stimulus–secretion mechanism for neutrophils, platelets and mast cells has been proposed [118, 121]. It is possible that products of the cycloxygenase pathway released from stimulated neutrophils may by feedback regulation control this stimulus–secretion coupling mechanism, although neutrophils are not major producers of prostaglandins. In the neutrophil the lipoxygenase pathway is the major eicosanoid pathway and its major product LTB_4 is a potent ionphore and chemotactic agent [122, 123]. In contrast to PGs LTB_4 raises cyclic GMP levels in neutrophils and elevated cyclic GMP levels have been associated with the release of lysosomal enzymes from the cell [124]. Macrophages may have a different stimulus–secretion mechanism than neutrophils, platelets and mast cells since although PGE_1, adrenaline and dibutyl-cyclic AMP has been shown to retard the release of lysosomal enzymes from macrophages their effect is only minor [115].

Tissue culture studies have produced useful information on the role of PGs in arthritic joints. Studies with normal synovium and rheumatoid synovium in culture have shown that rheumatoid synovium secretes more PGEs and collagenses per unit mass than normal synovium [125]. However, when synovial cells from both rheumatoid and non-rheumatoid synovium were dispersed with enzymes and subcultured, using repeated changes of culture medium to remove non-adherent cells, the adherent cells from both rheumatoid and non-rheumatoid synovium produced identical basal levels of PGEs and collagenase [126]. Moreover, both sets of cells looked the same and contained cells with dendritic (stellate) [127] morphology. Collagenase and PGE production was activated to the same extent in both sets of cells by the addition of either a soluble mononuclear cell factor (MCF) [128] derived from mononuclear blood cells or a synovial factor (SF) concentrated by ammonium sulphate precipitation from fragment cultures or either normal or rheumatoid synovium [125, 126]. A similar stimulating effect by MCF and SF was found on PGE production from trabecular bone cells and on PGE and plasminogen activator production in human articular chondrocytes [129, 130]. Both the production of MCF and SF and the expression of their stimulating action on synovial cells was inhibited by glucocorticoids [131]. The production of collagenase and PGEs by synovial cells after stimulation with either SF or MCF are probably independent events since PGE synthesis was inhibited by indomethacin but not that of collagenase [129]. However, PGE_2 is required for collagenase biosynthesis [132]. In experiments where the synthesis of collagenase from endotoxin stimulated macrophages was blocked by indomethacin this inhibition was overcome by the addition of small amounts of PGE_2 [133]. It therefore appears that PGE_2 has a promoting role in the production of collagenase. The source of SF in the cultures was probably the macrophage-type A cell which occurs in both normal and rheumatoid synovium [134]. The A cell type proliferates in synovium as rheumatoid disease develops and pannus formation commences. However, in normal synovium the A cells have physiological function as phagocytes. Therefore normal tissue as well as diseased tissue contains cells which given an appropriate stimulus can participate in events leading to joint destruction.

Collagenase is not the only proteinase produced from synovial tissue in culture. Explants of human synovium from normal, rheumatoid and osteoarthritic patients produce three metallo-proteinases (collagenase, gelatinase and proteoglycanase) [135] either in active or latent forms. Explants of normal or non-inflamed osteoarthritic synovium also produce a tissue inhibitor of metallo-proteinases (TIMP) in the early stages of the culture [135]. This inhibitor may be involved in the control of collagen resorption *in vivo* [136]. When a comparative study [126] was made of the amounts of TIMP and total collagenase

(free and latent) produced by normal and rheumatoid synovia in culture it was found that normal synovia produced TIMP but very little collagenase. In contrast rheumatoid synovia produced no detectable TIMP but considerable amounts of collagenase. When hydrocortisone was added to the cultures at various concentrations the steroid stimulated the formation of TIMP in the normal synovium. At a high dose it stimulated the formation of TIMP in the rheumatoid synovium and depressed collagenase production. Indomethacin depressed the formation of TIMP in normal synovia and collagenase production in the rheumatoid synovia. Both hydrocortisone and indomethacin depressed PGE synthesis in both cultures [126, 137]. The findings above suggest that a balance between production of collagenase and the production of TIMP may determine the extent of destructive processes in arthritis. Factors such as SF or MCF released from stimulated inflammatory cells and possibly normal synovial cells stimulate the production of PGs and proteinases by synovial cells; chondrocytes and possibly by bone cells. Corticosteroids by stimulating TIMP production in normal and rheumatoid synovia may contribute to an endogenous control mechanism while PGEs appear to promote both the production of inhibitor and collagenase [126, 138]. PGEs also have a modulating function in macrophage–lymphocyte interactions and they inhibit lymphocyte T cell proliferation and the release of lymphokines [139]. Activated macrophages secrete PGEs and monokines, including Interleukin-1 (Il-1) which has similar properties to MCF. Interleukin-1 is a lymphocyte activating factor found in rheumatoid synovial fluid [140] and it stimulates T-cell lymphocytes to produce lymphokines including a T-cell growth promoting factor known as Interleukin-2 [139]. Another lymphokine released stimulates macrophages to produce PGE_2 [139]. However, PGE_2 has a modulating effect at several points of control in this system. It suppresses the production of both monokines and lymphokines by feedback regulation. It also directly activates T-suppressor cells. Therefore, PGE_2 has a net inhibitory action on lymphocytes by suppressing lymphocyte proliferation and lymphocyte function [141]. Morley [142] proposed that the chronicity of rheumatoid arthritis may be due to a defective reactivity of lymphokine secreting T cells to the inhibitory effects of macrophage PGE_2.

Leukotrienes may also have immunoregulatory functions. Lipoxygenase inhibitors such as the free radical scavengers *p*-phenanthroline and gallic acid inhibit mitogen induced lymphocyte proliferation [143]. The mode of action of the leukotrienes is different from that the PGEs. The PGEs probably exhibit their immunoregulatory functions by raising intracellular cyclic AMP levels [144]. Leukotrienes appear to raise cyclic GMP levels [122]. Little is known about the immunoregulatory properties of the leukotrienes but LTC_4 and LTD_4 activate suppressor T cells [145]. A rich source of leukotrienes are the basophils but they have also been found in peritoneal macrophages [146].

A fourth messenger of the monokine–lymphokine type was discovered after a finding that when porcine cartilage and synovial tissue were co-cultivated a factor released from the synovial tissue caused the cartilage to reabsorb [147]. This factor was subsequently identified as a small acidic protein 'catabolin' which can also be produced from lymphocytes and monocytes stimulated by plant lectins [148, 149]. The probability that catabolin induces the resorption of cartilage was deduced from experiments where living and dead (by freezing and thawing) cartilage was cultured in the presence of catabolin containing medium prepared from normal synovial tissue. Cartilage breakdown was measured by the release of proteoglycan into the medium. Proteoglycan release was only evident when living cartilage was present. The dead cartilage gave similar values to the unstimulated controls. This rules out the possibility that the proteoglycan release was due to extracellular proteinases. The relationship of catabolin to other monokine and lymphokine messengers and whether its function is controlled by PGEs is unknown.

The production of monokines and lymphokines in diseased joints may explain some of the systemic effects observed in rheumatoid disease and in particular how other joints are involved. As the disease develops in the joint there is likely to be a build-up in levels of these intercellular messengers which may escape from the joint by lymphatic drainage and stimulate receptive cells in distant tissues.

Anti-oxidants and free radical scavangers

The blue alpha-2-glycoprotein caeroplasmin is present in plasma at a concentration of about 300 mg%. It consists of up to six or seven copper atoms bound to a single polypeptide chain. It is the chief copper transporting protein in blood [150] but also shows enzymic activity as a 'ferroxidase' and can catalyse the oxidation of ferrous iron to ferric. It also acts as a circulating anti-oxidant and can inhibit the auto-oxidation of lipids by ascorbic acid or inorganic iron [151]. Caeroplasmin is an acute phase reactant and is the most important circulating scavenger of superoxide [152]. Its scavenging effect against superoxide can be mimicked by a variety of copper containing compounds such as copper complexes with penicillamine [153], and cupric peptides [154]. Cupric and nickel complexes of macrocyclic polyamine derivatives are powerful scavengers of superoxide [155]. Caeroplasmin does not catalyse a dismutation action like superoxide dismutase but acts an anti-oxidant in a catalytic and stoichiometric manner similar to cytochrome *c* [152]. Catalase and glutathione are intracellular scavengers of hydrogen peroxide and therefore they prevent the formation of other activated oxygen species from superoxide anion. Glutathione removes hydrogen peroxide by the glutathione–peroxidase–glutathione reductase system using NADPH as a co-factor [156]. NADPH is regenerated by the hexose monophosphate shunt. Glutathione and catalase are both important in protecting cells from the toxic effects of hydrogen peroxide and derived oxygen species. They probably protect different subcellular structures. Neutrophils are particularly vulnerable to self destruction by generated oxygen species [157]. Many other anti-oxidants and free radical scavengers exist in biological systems such as the tocopherols, thiol compounds such as cysteine and other amino acids, e.g. histidine,

and tryptophan. Other compounds have been previously described in this commentary. Many exogenous compounds have been shown to have similar actions *in vivo* such as mannitol, chlorpromazine and some non-steroidal anti-inflammatory drugs. When used in sufficient quantities many of these exogenous and endogenous compounds show anti-inflammatory activity against animal models. Whether many of the endogenous anti-oxidants perform this function to any appreciable extent *in vivo* is doubtful since with the possible exception of ascorbic acid [158] they are not normally present in high concentrations. Ascorbic acid by reducing Fe^{3+} to Fe^{2+} may enhance the iron catalysed Haber–Weiss reaction [158]. The scavenging properties of compounds may explain the anti-inflammatory activity against animal models shown by a large number of endogenous compounds when used at high concentrations. Many amino acids such a L-tryptophan, phenylalanine, cysteine, valine, aspartic acid, alanine, glycine, glutamine, creatine and creatinine have all shown anti-inflammatory activity against animal models [159] but only in doses in excess of their physiological levels.

In conclusion inflammation occurs in two main phases. The early acute phase is a vascular response to injury with fluid exudation and the emigration of neutrophils into the damaged area. The second phase is dominated by the macrophage and is a phase of resolution and repair and the replacement of damaged tissues by fibrous tissue. Chronic inflammation occurs when the acute phase in inflammation is unsuccessful in eliminating the inflammatory trigger. Endogenous anti-inflammatory mechanisms probably have a number of functions. They protect tissues against toxic substances liberated during the inflammatory response. They control the repair and replacement of tissues damaged during inflammation and they may regulate the inflammatory response itself in relation to the nature and force of the inflammatory stimulus. A knowledge of these defensive systems offers the possibilities of developing new types of drugs for the treatment of inflammatory disease in the future.

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