PROSTAGLANDINS AND LYMPHOKINES IN INFLAMMATION

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SUMMARY

It is suggested that the inflammatory process in rheumatoid arthritis (RA) results from activation of small lymphocytes to produce lymphokines, whose biological properties are appropriate to a mediator of chronic inflammation. Since prostaglandins (PGs) and allied products of arachidonic acid metabolism have properties pertinent to chronic inflammation, these compounds can also be regarded as potential mediators of chronic inflammation, a viewpoint supported by the capacity of steroids and non-steroidal anti-inflammatory drugs (NSAIDs) to suppress PG formation. Certain observations are inconsistent with PGs fulfilling this role. These anomalies may be resolved by considering PGE$_2$ formation by macrophages to be a device for regulating lymphocyte activation.

A defect of lymphocyte reactivity to PGE$_2$ provides a basis for chronicity and has been demonstrated in multiple sclerosis (MS). Alternatively, should lymphocytes in lesions of RA be susceptible to PGE$_2$ inhibition, one consequence of NSAID treatment may be exacerbation of joint destruction.

There is abundant evidence that rheumatoid arthritis (RA) and allied diseases exhibit features of immunological dysfunction (Dumonde, 1976). Whilst it seems likely that both immune complexes and mechanisms of cellular immunity participate in the immunopathology of these diseases (Panayi, 1978), it is possible to propose that cellular immune mechanisms are primary events in the pathogenic process.

The evidence in favour of this proposition is, for the most part, circumstantial in nature, but nonetheless carries weight. Firstly, RA can be observed in patients who are seronegative with respect to the rheumatoid factor (Cats and Hazeroet, 1970). Secondly, in congenital agammaglobulinaemia, a polyarthritis can develop in a high proportion of individuals (Rotstein and Good, 1962). Thirdly, histological examination of synovial villi reveals abundant small lymphocytes (Glynn, 1972), with a high proportion of T-cells (Frøland, Natvig and Husby, 1973). Fourthly, lymphokines, the products of lymphocyte activation, have biological properties appropriate to mediators of chronic inflammation (Bray and Morley, 1977), and have been reported to be produced by synovial tissue \textit{in vitro} (Stastny \textit{et al.}, 1973). Fifthly, thoracic duct drainage can result in striking remission in severe RA, a process which is dependent upon the removal of the cell content of the lymph (Paulus \textit{et al.}, 1973). It is this latter study which provides the only direct experimental evidence that lymphocytes can initiate inflammation, since these workers showed that reinjection of thoracic duct lymphocytes to the quiescent joint results in severe exacerbation of the inflammatory process. Finally, by proposing the lymphocyte as the primary agency, it becomes possible to relate both lymphocyte and macrophage activation with the over-production of lymphokines, lysosomal enzymes and prostaglandin E$_2$ (Bray, Gordon and Morley, 1974).

On this basis, it is not unreasonable to consider lymphokines as putative mediators of several facets of the inflammatory response (Table I). These materials are particularly attractive by virtue of their capacity to initiate DNA synthesis in lymphocytes, to activate macrophages \textit{in vitro} and their ability to affect capillary endothelium \textit{in vivo} (Franco, Kelly and Morley, 1978). However, it is also necessary to attempt to relate the activity of lymphocytes to production of other mediators including prostaglandins.
TABLE I
LYMPHOKINES AND THE INFLAMMATORY RESPONSE

<table>
<thead>
<tr>
<th>Features of inflammation</th>
<th>Lymphokine</th>
<th>Potential mediator</th>
<th>Complement component</th>
<th>Lysosomal enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte activation</td>
<td>Mitogenic factor (MF)</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Macrophage activation</td>
<td>Macrophage activating factor (MAF)</td>
<td>—</td>
<td>C3b</td>
<td>—</td>
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<tr>
<td>Fibroblast activation</td>
<td>—</td>
<td>PGE1a</td>
<td>C3, C5, C567</td>
<td>—</td>
</tr>
<tr>
<td>Neutrophil accumulation</td>
<td>Leucocyte chemotactic factor (LCF)</td>
<td>Hete,</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Monocyte accumulation</td>
<td>Monocytic chemotactic factor</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Platelet accumulation</td>
<td>Platelet inhibition factor</td>
<td>Thromboxane A2, PGG2</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Cytotoxicity</td>
<td>Lymphotoxin</td>
<td>—</td>
<td>Terminal component</td>
<td>—</td>
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<tr>
<td>Bone resorption</td>
<td>Osteoclast activating factor (OAF)</td>
<td>PGE1a</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Collagen dissolution</td>
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<tr>
<td>Proteoglycan dissolution</td>
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<tr>
<td>Increased blood flow</td>
<td>Skin reactive factor (SRF)</td>
<td>PGE1a, PGE2a</td>
<td>C2-kinin</td>
<td>Leucocyte kininogenase</td>
</tr>
<tr>
<td>Increased vascular</td>
<td>Inflammatory factor (IF)</td>
<td>—</td>
<td>C3, C5*</td>
<td>—</td>
</tr>
<tr>
<td>permeability</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hyperalgesia</td>
<td>—</td>
<td>PGE2a</td>
<td>Arachidonic acid</td>
<td>—</td>
</tr>
</tbody>
</table>

(PGs) (and related products of arachidonic acid metabolism), lysosomal enzymes and complement components (Table I).

The evidence favouring involvement of PGs in chronic inflammation is substantial. PGE2 is a potent vasodilator agent and it is probably a consequence of this property that it is able to indirectly modify vascular permeability (Williams and Peck, 1977). PGE2 similarly appears to have an indirect effect in pain production, being markedly hyperalgesic (Ferreira, 1972). In addition to these acute effects, which have been subject to extensive study by pharmacologists, there are other properties of PGs of considerable relevance to chronic inflammation, namely stimulation of bone resorption (Harris et al., 1973) and fibroblast activation (Peters et al., 1974). It is nevertheless clear that the PGs are relatively limited in their capacity to effect many of the features of the inflammatory response and it remains to be established whether short-lived intermediate compounds such as the endoperoxides (PGH2 and PGG2), prostacyclin (PGI2) or thromboxane A2 can become established as agents of superior potential to PGE2. In the case of PGE2 there is now substantial evidence that it is produced by synovial tissue.
in RA both \textit{in vitro} (Robinson, Smith and Levine, 1973) and \textit{in vivo} (Levine, 1973). When these observations are considered in light of the established capacity of both NSAIDs (Flower, 1974) and glucocorticosteroids (Bray and Gordon, 1976) to suppress PG formation, it is not surprising that PGs have become widely accepted as inflammatory mediators, whose sustained production accounts for the signs and symptoms of an inflammatory response.

However, there are certain anomalous observations which suggest that this role as a classical inflammatory mediator is inappropriate. In experimental studies, it has been observed that chronic inflammatory diseases can be more severe in animals made deficient in polyunsaturated fatty acids by dietary means, despite the absence of PGs and related compounds (Clausen and Mailer, 1967). Conversely, administration of PGE$_9$ has been reported to have a suppressive effect on rat adjuvant arthritis (Bonta et al., 1977). The clinical counterpart of these experimental observations is the appreciation that compounds reported to be potent PG synthesis inhibitors have proved to be poorly effective in RA (Sturge et al., 1977), so that aspirin can continue to be regarded as a primary drug of choice in RA (Mills, 1974), despite its relatively low potency as a PG synthetase inhibitor in inflammatory cell populations compared to other compounds (Bray and Gordon, 1978). Thus, to regard the inflammatory process as primarily due to over-production of E-type PGs (Ferreira and Vane, 1974) or allied compounds (Kuehl, et al., 1977) is a viewpoint inconsistent with both clinical and experimental experience, notwithstanding the evidence that therapeutic concentrations of most NSAIDs suppress arachidonic acid metabolism.

Investigations of the possible association between lymphokines and prostaglandins have revealed a relationship which can accommodate these anomalous observations. In early studies, it was evident that the PGs, particularly PGE$_1$ and PGE$_2$, were not noteworthy for their inflammatory potential (Horton, 1963), but were clearly capable of modifying the response of cells to other agents by virtue of their considerable potency as activators of adenylate cyclase (Bergström, 1967). In fact, it seems likely that much of their inflammatory potential arises from a capacity to intensify responses to other inflammatory mediators as has been clearly demonstrated for pain production (Ferreira, 1972) and for increased vascular permeability (Williams and Peck, 1977). These regulatory effects of PGs are not restricted to effector cells responding to inflammatory mediators but are also observed at the level of mediator secretion, a phenomenon consistent with the capacity of E series PGs to regulate secretion in diverse cell types presumably by actions on the second messengers Ca$^{++}$ and cAMP (Rasmussen and Goodman, 1977). Since it was already well established that PGE$_1$ inhibited lymphocyte activation (Smith, Steiner and Parker, 1971), it is not exceptional that this should be accompanied by an inhibition of lymphokine secretion (Bray et al., 1974). This latter observation achieves greater significance in light of the unexpected finding that the generation of lymphokine in mixed cell populations was accompanied by the formation of PGE$_2$ and that the origin of the substantial levels of PGE$_2$ observed was the macrophage (Gordon, Bray and Morley, 1976). Since lymphokines can stimulate both PGE$_2$ production and lysosomal enzyme secretion (Pantelone and Page, 1975) and macrophages can be shown to generate and cleave the C3 component of complement (Schlormlemmer and Allison, 1976), it is possible to relate over-production of PGE$_2$, lymphokines, lysosomal enzymes and the complement components C3a and C3b to a single event, lymphocyte activation (Morley, 1976). This can be viewed as a physiological event or as providing a basis for chronicity, since lymphokines provide a positive feedback system...
for the recruitment and activation of additional lymphocytes (Fig. 1), whilst C3b provides a similar positive feedback system at the level of the macrophage, to give a basis for chronicity not involving lymphocyte persistence.

Such a regulatory role for PGE₂ has interesting consequences. It is possible that defective reactivity to PGE₂ is a feature underlying, or at least reflecting, chronicity of inflammatory responses (Morley, 1974). This possibility has been investigated in hospitalized patients with active multiple sclerosis (MS). In contrast to normal individuals, these patients exhibit leucocyte migration inhibition in vitro that cannot be overcome by PGE₂ (Kirby et al., 1976; Kirby, 1978). This is unlikely to be pathognomonic for MS as similar results were obtained in a small series of patients with CFA (Kirby, unpublished observations) as well as in certain neurological control patients. It is of interest to determine if this is a feature of lymphocyte behaviour in RA. In a small series of patients defective reactivity to PGE₂ was only observed at high PGE₂ doses (1 μg/ml) (Kirby, unpublished observations). Other investigators of this phenomenon have also not resolved this question for whilst Zurier, Doty and Goldenberg (1977), using adenyl cyclase activation as a response parameter, observed defective reactivity in synovial lymphocytes, they reported peripheral blood lymphocytes in the same patients to have normal reactivity. On the other hand Dayer et al. (1976), studying RA synovial fragments in vitro observed an effect of indomethacin which suggests normal PGE₂ reactivity.

It is this latter observation that is of particular relevance to NSAID action in RA. A prediction from Fig. 1 is that when PG formation is suppressed, the generation of lymphokines should be enhanced. This phenomenon can be observed in peripheral blood leucocytes of normal individuals (Gordon et al., 1976). The observation of Dayer et al. (1976) that whilst stimulation of PGE₁ in synovial fragments by products of

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**Fig. 1. The lymphocyte—macrophage axis.**
lymphocyte activation (i.e. lymphokines) could be suppressed by indomethacin the concomitant collagenase generation was paradoxically enhanced, is consistent with this prediction.

The clinical consequences of these relationships is not without interest. The NSAIDs seem likely to have limited efficacy, for whilst they are potent inhibitors of cyclo-oxygenase (Youlten, 1978), they do not limit lymphocyte or macrophage activation. The suppression of the vascular and pain-producing manifestation of PG formation are of immediate subjective relevance to both the patient and the physician. However, it would appear possible that concurrent with the symptomatic relief there may be an exacerbation of the process of joint destruction.

The emphasis that is placed upon PG synthesis inhibition in the newer NSAIDs is disconcerting for it appears quite possible that emphasis of this particular aspect of NSAID action may result in the selection of compounds of less long-term benefit to the patient than existing drugs. It is hoped that rheumatologists will seek to refute (or confirm) this prediction.

REFERENCES
—— ——— (1976) "Regulation of lymphokine secretion by prostaglandins". Agents & Actions 6, 171-5.
FRANCO, M. D., KELLY, R. H. and MORLEY, J. (1978) "Comparison of haematogenous cell infiltrate evoked by lymphokine injection with that of delayed hypersensitivity reactions". Amer. J. Path. (In press.)


DISCUSSION

**Husby:** Do you think that the PGE can act to depress the lymphocytes itself or do you think that there is an actual need for a second messenger such as cyclic AMP?

**Morley:** I think it is quite possible that E type prostaglandins might act quite independently of cyclic AMP and this is why I would say that the evidence for a PG receptor is not at all convincing. We spent two years looking at this problem but could not find any experimental evidence.

**Husby:** I believe that the rheumatoid synovial cells can act without prostaglandins. I am therefore stressing the protecting lymphocytes in the synovial tissues.

**Morley:** Well, this is just one thing prostaglandin might do in the synovial cavity, but I agree with you both from our observations and from the literature that the synovial tissue in rheumatoid subjects is a very good source of E type prostaglandin, probably PGE₂. I did not mention that we now have good chemical evidence from gas liquid chromatography and mass spectrometry that the material that we are looking at is PGE₂.

**Grahame:** If one arranges the relative potency of the commonly used anti-inflammatory drugs in terms of their ability to inhibit prostaglandin synthetase and relates that to their clinical efficacy, there seems to be very little association or correlation between the two. Does this imply that prostaglandin synthetase inhibition is not an important factor in the efficacy of these drugs?

**Morley:** Of course it is difficult to rank them in order of clinical efficacy because of differing opinions. You can, however, relate them to the relative dose that is given. There is clear correlation between the ability of these drugs to inhibit prostaglandin biosynthesis by macrophages and their dosage. I would suggest that they do have this property which is a necessary factor in the symptomatic relief in terms of pain and vasodilatation but they must have some other properties.