Eosinophils in eosinophilic endomyocardial disease

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The idiopathic hypereosinophilic syndrome is a disease which mainly affects young men (Spry, 1982). It is thought to be either the result of an acquired genetic defect in eosinophils themselves, or the consequence of an excessive production of an eosinopoietic factor (Slungard et al., 1983; Metcalf et al., 1983), but neither mechanism has yet been fully defined. Many of these patients present with the general clinical features of the hypereosinophilic syndrome (Spry et al., 1983a), including thromboembolic disease, coughing attacks and skin lesions, but over 50% present with breathlessness and congestive cardiac failure as a result of eosinophilic endomyocardial disease (Davies et al., 1983). Most of the patients, who do not have endomyocardial disease at presentation, develop it later so that the final incidence approaches 85% (Spry et al., 1983b).

This review describes recent work which was designed to help in the diagnosis of eosinophilic endomyocardial disease in its early stages, and to determine the pathogenesis of the endocardial damage. It was hoped that this might lead to the development of new methods to inhibit the course of the disease, which often leads to intractable heart failure which may result in death.

Clinical studies

The late stage of eosinophilic endomyocardial disease gives rise to pulmonary oedema causing breathlessness and widespread shadowing in chest radiographs (Davies et al., 1983; Parrillo et al., 1979). As this may be associated with symptoms due to the hypereosinophilic syndrome itself (fever, malaise and weight loss), it can be misinterpreted as a respiratory infection, but patients usually respond, at least temporarily, to diuretics. On the right side of the heart, eosinophilic endomyocardial disease (which is always bilateral) gives rise to a raised jugular venous pressure, dependent oedema, and ascites. At this late stage, the diagnosis is confirmed by echocardiography (Davies et al., 1982; Borer et al., 1977). It is rarely necessary to carry out cardiac angiography, until a decision is needed about possible surgery, with valve replacement and/or endocardectomy (Davies et al., 1981). Unfortunately the earlier stages of the disease cannot be diagnosed at the bedside or by using echocardiography. For this reason endocardial biopsy has become the method of choice for diagnosing the early necrotic and thrombotic stages of the disease (Spry et al., 1983b; Kim et al., 1984). As the outflow tract is not involved, the biotome has to be positioned with some care to avoid sampling the normal endocardium. In view of the potential risk of embolism from mural thrombus in the left ventricle, it is usually advisable to restrict these biopsies to the right ventricle.

Eosinophil biochemistry

The presence of many degranulated eosinophils (Spry & Tai, 1976) in the blood of patients who have (or will develop) eosinophilic endomyocardial disease (Jaski et al., 1978; Spry et al., 1985) has supported the view that eosinophils themselves might be involved in the pathogenesis of this condition. Recent work on the biochemistry of eosinophils lends support to this suggestion. Eosinophil granules contain (besides lysosomal granule enzymes which are common to other inflammatory cells), four basic proteins (Ackerman et al., 1983; Klebanoff et al., 1983) which may be the basis for the capacity of eosinophils to kill parasites and damage mammalian tissues. The properties of these proteins are shown in Table I. Eosinophil cationic protein (ECP) has been found to kill isolated rat heart cells at $1 \times 10^{-2} - 1 \times 10^{-6}$ M (Tai et al., 1982). It appears to do this by interfering with $\text{Na}^+$ transport in the plasma membrane and preventing mitochondrial respiration by a direct inhibitory effect on pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. ECP can also affect the coagulation system by an effect on factor XII (Venge et al., 1979) and other coagulation proteins (Dahl & Venge, 1979). Recently it has been found that eosinophils produce large amounts of platelet activating factor (Lee et al., 1984). This may account for the high incidence of thrombosis and thromboembolic complications in

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Table I  Properties of human eosinophil granule basic proteins

<table>
<thead>
<tr>
<th>Granule proteins</th>
<th>Molecular weight (× 10^3)</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophil peroxidase (EPO)</td>
<td>75</td>
<td>Toxic to schistosomula, trypanosomes, and tumour cells</td>
</tr>
<tr>
<td>Eosinophil derived neurotoxin (EDN)</td>
<td>19</td>
<td>Toxic to brain tissues in animals – the Gordon phenomenon</td>
</tr>
<tr>
<td>Eosinophil protein-X (EP-X)</td>
<td>19</td>
<td>Identical to EDN?</td>
</tr>
<tr>
<td>Eosinophil cationic protein (ECP)</td>
<td>16</td>
<td>Toxic to schistosomula, and heart cells. Affects coagulation, T-cell responses and produces the Gordon phenomenon</td>
</tr>
<tr>
<td>Eosinophil major basic protein (MBP)</td>
<td>12</td>
<td>Toxic to schistosomula, bronchial cells, etc.</td>
</tr>
</tbody>
</table>

patients with the hypereosinophilic syndrome (Spry et al., 1983a; Chaine et al., 1982). Eosinophil major basic protein (MBP) has also been shown to damage mammalian tissues, especially the respiratory epithelium (Frigas et al., 1980; Davis et al., 1984). The eosinophil neurotoxin (EDN) (Durack et al., 1981) and eosinophil protein-X (EP-X) (Peterson & Venge, 1983) are closely related or identical proteins which cause demyelination after injection into the cerebrospinal fluid of experimental animals. Eosinophil peroxidase (EPO), which is distinct from myeloperoxidase, is also capable of damaging and killing tumour cells, bacteria and large organisms by generating hypochlorite. Comparative studies on the capacity of these effector mechanisms suggest that ECP and EPO are the most toxic of the eosinophil basic proteins (Gleich & Loegering, 1984; Butterworth et al., 1979; McLaren et al., 1984). Experiments to examine the apparently marked susceptibility of endocardial cells to injury has not been possible, as these cells cannot be isolated from other myocardial cells. Also, until recently, it has been difficult to purify the eosinophil granule basic proteins because of their strong positive charge, and their capacity to bind to surfaces and membranes, including filters for sterilizing solutions. This has made it difficult to study the effects of highly purified proteins at low concentrations in vitro or in vivo. However, the introduction of ion-exchange columns with uniform characteristics, combined with fast protein liquid chromatography (FPLC), has made their purification much easier. This means that experiments using highly purified eosinophil granule proteins can now be carried out more easily.

Assays for granule proteins

Specific rabbit antibodies to ECP (Venge et al., 1977) and MBP (Wassom et al., 1981) have been prepared. Also, in the last few years, monoclonal antibodies have been raised to each of the principal eosinophil granule proteins (Tai et al., 1984a). This has enabled radioimmunoassays and radio-immunometric assays to be carried out. A number of patients with the hypereosinophilic syndrome and tumour-induced eosinophilia have been shown to have raised serum ECP (Spry et al., 1985) and MBP levels (Wassom et al., 1981), but the development of heart disease was not directly correlated with raised levels. For this reason, studies were done to see whether methods to detect ECP and MBP in endocardial biopsies, and cardiac tissues taken at post-mortem, could provide useful diagnostic information, and further evidence for the involvement of these basic proteins in the pathogenesis of endocardial damage in patients with hypereosinophilia.

Formalin-fixed and paraffin-embedded cardiac tissues from 18 patients with the hypereosinophilic syndrome were examined by immunohistochemical techniques, using an affinity column-purified rabbit antibody which was specific for MBP (Filley et al., 1981), and a mouse monoclonal antibody which bound to the secreted forms of ECP and EP-X (Tai et al., 1984a). Three patients had cardiac biopsies from the right ventricle. Results demonstrated that these proteins were present in the endocardium, and in areas of acute inflammation, endocardial thrombi and occasionally in blood vessels close to areas of cardiac injury (Tai et al., unpublished). Deposits were most marked in the early necrotic and later thrombotic stages of the disease. An example of the endocardial deposition of ECP in a patient with a carcinoma of the lung-induced hypereosinophilia, who developed eosinophilic endomyocardial disease, is shown in Figure 1.

Conclusions

The results of these new clinical and immunological approaches to the study of eosinophilic heart disease, have shown that the disease can be diagnosed in its
early stages, before irreversible and clinically significant deposits of fibrous tissue have accumulated in the endocardium. This has also led to fresh insights into the relationship between the presence of eosinophils in the blood and tissues, and the development of heart disease, confirming the suggested role for eosinophils in this disease (Spry & Tai, 1984), as outlined in Figure 2.

However, a number of important issues remain unexplained. Firstly, it is not known why patients with the idiopathic hypereosinophilic syndrome have raised blood eosinophil counts. Secondly, it is not known whether eosinophils are activated in vivo by the same factors which are effective in vitro (Thorne et al., 1985), and how they are induced to degranulate (Tai & Spry, 1981). In addition, the specific localization of the inflammatory response in the endocardium is also not explained, although it has been suggested that cells in this part of the heart may have different metabolic requirements, or membrane constituents, as a result of their location in the inner parts of the heart, where the blood supply is different from other areas (Spry et al., 1983b).

An important practical question is whether there are any natural or synthetic inhibitors of eosinophil basic proteins which could be used to prevent the development, or the progression, of the heart disease. Theoretically, any strongly anionic substance can bind to these cationic proteins, and possibly inhibit their toxic effects. At present, heparin is the only one available for clinical use (Kierszenbaum et al., 1982), but it has not been shown to be beneficial in the short term in the few instances when it has been used in patients with cerebral thromboembolic disease (strokes). Chronic treatment with subcutaneous heparin, to prevent progression of the heart disease, has not yet been attempted. As endocardial disease takes several months or years to develop, there are practical problems with this approach. Steroids may be more effective, as they have the property of inhibiting eosinophil degranulation, at least in vitro. On the other hand, warfarin, possibly combined with dipyridamole, would be expected to reduce thromboembolic complications, which are a common cause of death. Once the factors responsible for inducing excess eosinophil production (Slungaard et al., 1983; Metcalf et al., 1983), and eosinophil activation (Thorne et al., in press) and degranulation (Tai & Spry, 1981), have been defined, more rational approaches to the prevention of this disease may be possible. Until then, increased awareness of the clinical association of endocardial damage with eosinophilic disorders, and the effectiveness of modern diagnostic

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**Figure 1** Immunocytochemical staining of the post-mortem endocardium of a patient with carcinoma of the lung-induced hypereosinophilia, and eosinophilic endomyocardial disease in the acute stage. Formalin-fixed and paraffin-embedded sections of the endocardium were stained with mouse monoclonal antibody EG2, using alkaline phosphatase-linked second antibodies, giving a red reaction product with fast red dye, and counterstained with haematoxylin (Tai et al., 1984a). Eosin was not used. Control sections were treated with an unrelated monoclonal antibody of the same isotype, and treated in the same way. Eosinophil cationic protein (ECP), and eosinophil protein-X (EP-X) were localized to the endocardium (small arrows). Monoclonal antibody EG2 also bound to these proteins in activated eosinophils, which were demonstrated to be present in the endocardium (large arrows). x 400.

**Figure 2** Suggested mechanisms for eosinophil-induced cardiac injury. Circulating eosinophils, which can be stained with the monoclonal antibody EG1 to eosinophil cationic protein (ECP), first become activated in the blood or tissues. Activated eosinophils contain the secreted form of ECP, and eosinophil protein-X (EP-X), which share a common antigenic epitope, and are now recognized by antibody EC2 (Tai et al., 1984). Activated eosinophils degranulate in the blood and/or tissues, and their toxic products, including ECP and the eosinophil major basic protein (MBP), injure the endocardial cells, leading to the development of areas of acute necrosis and later fibrosis.
techniques, will lead to further insights into the nature of eosinophilic endomyocardial disease. This, in turn, will provide further opportunities for work on the pathogenesis and treatment of the disease.

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References


