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Degranulated eosinophils, eosinophil granule basic proteins and humoral factors in Nigerians with endomyocardial fibrosis

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Summary

The frequency of eosinophilia, degranulated eosinophils, and raised serum levels of eosinophil granule basic proteins was determined in ten Nigerians with EMF and fifteen normal controls matched for age and sex, and from the same environment and social background as the patients. Sera from the patients and control subjects were also examined for auto-antibodies, immune complexes, filaria antibodies and immunoglobulins. A mild eosinophilia was observed in four of the patients. The mean direct eosinophil count in the patients ($0.407 \pm 0.14 \times 10^9$/l) was, however, not significantly different from that of the control subjects ($0.399 \pm 0.07 \times 10^9$/l). Degranulated eosinophils were absent in the blood and bone marrow aspirates of the patients, and peripheral blood of the controls. It was also found that the mean concentration of eosinophil granule basic proteins (ECP/EPX) in the patients (0.278 ± 0.1 μg/ml) was not significantly different from the mean value for the control subjects (0.371 ± 0.1 μg/ml, $P > 0.5$). Serological studies using *Brugia pahangi* antigens failed to demonstrate filaria antibodies in the patients and the control subjects. Microfilaria were also absent in their peripheral blood films. Using the indirect immunofluorescent technique, anti-heart antibodies were not demonstrated in the sera of the EMF patients and control subjects. Five (50%) of the patients and four (30.7%) of the normal controls had immune complexes levels far in excess of the upper limit of normal. However, the mean concentration of immune complexes in the patients (16.35%) was not significantly different from the value for control subjects (12.13%, $P > 0.5$). The mean concentrations of IgG and IgA (28.7 ± 3.9 and 2.8 ± 0.8 gm/l, respectively) in the patients were significantly higher than the mean values (19.5 ± 0.6 and 2.3 ± 0.2 g/l, respectively; $P < 0.05$) for the controls. These findings are similar to those recently reported in dilated cardiomyopathy (DCM) and rheumatic heart disease (RHD), and seem to supplement the increasing clinical evidence that EMF, DCM and RHD may be sequel to infection. It is concluded that degranulated eosinophils and significantly elevated serum levels of eosinophil granule basic proteins are not present in EMF seen in Ibadan, Nigeria. Eosinophilia is as common in the controls as in the patients. This study has shown no significant role for humoral immunity in the mechanism of cardiac damage in EMF.

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Résumé

On a déterminé la fréquence de l'éosinophilie, de l'ésinophile dégranulé et des niveaux élevés de serum de protéines de base de l'ésinophile en granules chez dix Nigerians atteints de l'EMF chronique et chez quinze sujets de contrôle normaux de même âge, du même sexe et du même milieu que les patients. On a examiné le sérum des patients et des sujets de contrôle particulièrement afin d'y déterminer la présence des aauto-anticorps, des complexes immunisants, des anticorps de filaire et des immunoglobulines. Chez quatre patients, on a observé une éosinophilie peu sévère. Toutefois le chiffre moyen direct de l'éosinophile de ces patients ($0.407 \pm 0.14 \times 10^9$/l) n'était pas très différent de celui des sujets de contrôle ($0.399 \pm 0.07 \times 10^9$/l). On a constaté l'absence de l'eosinophile dégranulé dans les aspirants du

sang et de la moëlle des patients aussi bien que dans le sang périphéral des sujets de contrôle. Cependant, la concentration moyenne des protéines de base de l'éosinophile en granules (ECP/EXP) des patients (0.278 µg/ml) n'était pas très différente de la valeur moyenne pour les sujets de contrôle (0.371 µg/ml, $P > 0.5$). Les études sérologiques utilisant les antigènes Brugia pahangi n'ont pas révélé la présence des anticorps de filaires ni chez les patients, ni chez les sujets de contrôle. Les vers microfilaires ne se trouvaient non plus dans le film de leur sang périphéral. D'après la technique immunofluoresçante, il n'y avait pas d'anticorps de coeur dans le sérum des patients atteints de l'EMF et des sujets de contrôle. Chez cinq patients (50%) et chez quattre sujets de contrôle (30.7% du groupe), le niveau des complexes immunisants était de loin supérieur à la limite extrême du normal. Toutefois la concentration moyenne des complexes imunisants chez les patients (16.35%) n'était pas très différente de celle pour les sujets de contrôle (12.13%, $P > 0.5$). Les concentrations moyennes d'IgG et d'IgA (28.7 ± 3.9 et 2.8 ± 0.8 gm/l, respectivement) chez les patients étaient beaucoup plus élevées que les valeurs moyennes pour les sujets de contrôle (19.5 ± 0.6 et 2.3 ± 0.2 gm/l, respectivement; $P < 0.05$). Ces résultats ressemblent à ceux rapporté pour la cardionypathie dilatée et la maladie rheumatique de coeur et semblent s'ajouter aux indications qui de plus en plus présentent l'EMF, le DCM et le RHD comme suite à une infection. La conclusion en est que l'éosinophile dégranulé et les niveaux élevés des protéines de base d'éosinophile en granules ne se présentent pas chez les cas d'EMF chroniques, examinés à Ibadan au Nigéria. La éosinophilie est aussi répandue chez les sujets de contrôles que chez les patients. Cette étude n'attribue aucun rôle significatif à l'immunité humorale dans le mécanisme des dégâts cardiaques chez les cas d'EMF.

Introduction

A great deal is now known about endomyocardial fibrosis (EMF) since its original description by Bedford and Konstam in 1946. Its pathological, clinical, echocardiographic, haemodynamic and angiographic features are well defined (Davies & Ball, 1955; Falase, 1983; World Health Organization, 1984), but its aetiology remains unknown. Many theories abound (Falase, 1983) but the most promising is the link between activated eosinophil and endomyocardial damage (Olsen & Spry, 1979; Andy, Bishara & Soyinka, 1981; Spry, Tai & Davies, 1983).

An association between eosinophilia and EMF has long been recognized. Twelve of the twenty-four patients in Davies' (1984) original description of EMF showed a significant eosinophilia; six of the sixteen patients described by Connor *et al.* (1967, 1968) also had eosinophilia. Brockington, Olsen & Godwin (1967) found intense eosinophilia in Europeans who developed EMF while working in the tropical zones, while Ive *et al.* (1967) drew attention to the association between EMF, filariasis and eosinophilia. French and Belgian workers have also found a similar association in their patients with EMF (Parry, 1976). In 1979, Jaiyesimi, Onadeko & Antia described four patients with EMF, each of whom had *Mansoni* schistosomiasis, dermatosis and eosinophilia. An association between tumour-induced eosinophilia and EMF has also been described in several reports (Brockington, Luzzatto & Osunkoya 1970; Roberts, Buja & Ferrans, 1970; Manthorpe *et al.*, 1977).

Loffler in 1936 described a disease characterized by endomyocardial damage and high levels of eosinophils in the blood and tissues, but for a long time, Loffler's endomyocardial disease and EMF were considered to be separate entities. EMF was believed to be confined to the tropical regions and Loffler's endomyocardial disease to the temperate zone. However, in 1973, Brockington and Olsen reviewed pathological specimens of both diseases and found that the lesions were indistinguishable. Based on this observation, Olsen and Spry (1979) suggested that a similar pathogenetic mechanism may be responsible for the development of tropical EMF. This view was reinforced by the report of Andy *et al.* (1981), who found the presence of eosinophilia in forty-four Nigerians with recent onset obscure heart disease. Thirteen of the forty-four patients had filariasis. The eosinophilia regressed after treatment with diethylcarbamazine but nine developed features of EMF during a 2-year follow-up. Recently, Spry *et al.* (1983) found that degranulated forms of eosinophils

were present in the blood and tissues (including the endomyocardium) of eleven patients with Loffler's endomyocardial disease. Nine of the eleven also had raised levels of eosinophil granule basic proteins (ECP/EPX). Spry *et al.* (1983) also showed that the eosinophil granule basic proteins were toxic to isolated rat heart cells *in vitro* and this led them to conclude that eosinophils may degranulate under certain conditions, releasing toxic granule basic proteins that damage the heart, causing endomyocardial disease.

Since EMF is prevalent in the rain-forest zone of Nigeria, we undertook this study to determine the frequency of degranulated eosinophils and eosinophil granule basic proteins in Nigerians with EMF, and also to assess the role of humoral immunity in the disease process.

Patients and methods

Ten Nigerians with EMF were studied. A diagnosis of EMF was made after clinical evaluation and angiocardiographic studies. Fifteen normal individuals from the same environment and social background as the patients, and matched for age and sex, served as controls. Blood was collected from each patient and controls for haematological and immunological studies. Each patient also had a bone marrow examination.

A thin blood film obtained from each patient and control was stained with Leishman's reagent and examined under high magnification for degranulated eosinophils. Slides of fresh preparation (thin and thick films) of peripheral blood, taken between 10.00 h and 15.00 h, and between 21.00 h and 24.00 h were also examined under low and high power magnification for microfilaria worms. An absolute eosinophil count was made in each blood sample by the method of Discombe (1946).

Serum immunoglobulins were determined by the single radial immunodiffusion technique (Fahey & McKelvey, 1965) and immune complexes by the modified ^{125}I-Clq binding test (Zubler *et al.*, 1976). Filaria antibodies were detected by immunofluorescence technique using cat blood infected with *Brugia pahangi* microfilaria as antigen. Eosinophil granule basic proteins (ECP/EPX) were assayed as described by Spry *et al.* (1983), while autoantibodies in the sera were detected by the modified indirect immunofluorescence technique (Maisch *et al.*, 1982).

Results

Nine of the patients were male, one female. Their ages ranged from 8 to 57 years with a mean age of 20.7 years. Seven of them had right ventricular EMF and three biventricular EMF. The control subjects consisted of ten males, five females. Their ages ranged from 5 to 40 years, with a mean age of 16.5 years. The patient had a higher mean age than the controls, but the difference was not statistically significant ($P > 0.5$).

Eosinophil counts

Table 1 shows the direct eosinophil counts in the patients and controls. The mean eosinophil count in the patients was not significantly different from the mean value for the controls.

Table 1. Eosinophil counts in the EMF patients and control subjects

Patients			Controls	
No.	Direct eosinophil count × 10^9/l		No.	Direct eosinophil count × 10^9/l
1	0.080		1	0.200
2	0.400		2	0.800
3	0.440		3	0.510
4	0.240		4	0.128
5	0.040		5	0.760
6	0.510		6	0.220
7	1.587		7	0.180
8	0.120		8	0.160
9	0.100		9	0.072
10	0.560		10	0.114
			11	0.500
			12	0.470
			13	0.702
			14	0.620
			15	0.550
Mean	0.407	a*		0.399 b*
s.e.†	0.144			0.066

*a *v* b: $P = > 0.5$.
† s.e. = Standard error.

Degranulated eosinophils

Degranulated or vacuolated eosinophils were absent in the peripheral blood and bone marrow aspirates of the patients, and blood films of the control subjects.

Microfilaria worm in peripheral blood

No microfilaria worm was found in the peripheral blood of the patients.

Eosinophil granule basic protein (ECP/EPX)

Table 2 shows the ECP/EPX values in both the patients and control subjects. The mean concentration of ECP/EPX in the patients was lower than the mean for the controls but the difference was not statistically significant.

Filaria antibodies

Filaria antibodies were not detected in the sera of either the EMF patients or control subjects.

Auto-antibodies

Anti-heart, anti-nuclear, anti-smooth muscle and anti-mitochondrial antibodies were absent from the sera of the patients and controls.

Immunoglobulins

Table 3 summarizes the mean concentrations of the various serum immunoglobulins in the patients and controls. The mean serum concentrations of IgG and IgA in the EMF patients were significantly higher than the values obtained in controls. The serum IgM levels were not significantly different between the EMF patients and controls.

Immune complexes

Table 4 shows the values obtained in the EMF patients and controls. There was no significant difference in the mean values obtained in the two groups.

Table 2. Serum ECP/EPX levels in the EMF patients and control subjects

Patients	ECP/EPX (μg/ml)	Controls	ECP/EPX (μg/ml)
1	0.084	1	0.968
2	0.127	2	0.184
3	0.068	3	0.107
4	0.864	4	0.186
5	0.353	5	0.136
6	0.072	6	0.134
7	0.076	7	0.071
8	0.070	8	0.074
9	0.968	9	0.376
10	0.101	10	0.323
		11	0.392
		12	0.164
		13	0.496
		14	0.481
		15	1.480
Mean	0.278		0.371
	a*		b*
s.e.†	0.110		0.100

*a *v* b: $P > 0.5$.
†s.e. = Standard error.

Discussion

This study has shown a mild eosinophilia in four of the patients with EMF, but no significant difference existed between the mean direct eosinophil counts in patients and controls. Degranulated eosinophils were absent in the blood and bone marrow aspirates of the patients. The mean concentrations of eosinophil granule basic proteins (ECP/EPX) were not different in the two groups.

These findings are in agreement with the observations of Davies *et al.* (1983) who studied twenty-eight Indians with EMF and found that they had blood eosinophil counts that were no higher than those in age and sex matched controls. They also found no degranulated eosinophils in the peripheral blood of the patients and controls.

As stated earlier, studies on European residents in Africa who developed EMF (Brockington *et al.*, 1967), and on Nigerians with the disease (Andy *et al.*, 1981) have incriminated filariasis as the most frequent cause of eosinophilia seen in EMF. However, our serologic study, using *B. Pahangi* antigens failed to demonstrate filaria antibodies in the EMF patients and controls. Although the specificity and sensitivity of anti-filaria antigen for the diagnosis of microfilariasis are known to be low (Andy & Olusi, 1982), the absence of microfilaria worms in the peripheral blood of these

Table 3. Mean serum immunoglobulin concentrations in the EMF patients and control subjects

Immunoglobulin levels (g/l)	EMF patients (n = 10)	Controls (n = 15)	Difference: P value
IgG			
Mean	28.72	19.50	< 0.05
s.e.†	3.864	0.642	
IgM			
Mean	2.180	1.713	> 0.05
s.e.†	0.260	0.207	
IgA			
Mean	3.815	2.341	< 0.05
s.e.†	0.838	0.211	

†s.e. = Standard error.

Table 4. Serum immune complexes in the EMF patients and normal control subjects

Patients	Immune complexes (% positive)	Controls	Immune complexes (% positive)	
1	4.8	1	12.2	
2	23.4	2	26.9	
3	10.4	3	18.6	
4	4.9	4	43.9	
5	54.4	5	4.2	
6	15.5	6	9.2	
7	6.6	7	3.3	
8	14.1	8	5.9	
9	8.3	9	4.8	
10	21.1	10	3.0	
		11	3.4	
		12	6.2	
		13	16.0	
Mean	16.350		12.123	b*
s.e.†	4.697		3.321	

*a *v* b: $P > 0.50$.
†s.e. = Standard error.

patients further suggests that eosinophilia observed in four of the patients may be related to other causes, most probably intestinal helminths. Intestinal helminths accounted for the eosinophilia observed in 80% of EMF patients studied recently by Jaiyesimi, Salimonu and Anti (1984).

The differences between the eosinophil abnormalities observed in this study and those reported in Loffler's endomyocardial disease may be explained by taking into consideration the nature of the underlying disease process. In Loffler's endomyocardial disease, the patients present in the early necrotic stage when the

trigger of endocardial damage, the hypereosinophilic syndrome, is profusely active. Conversely, all the patients in this study were seen in an advanced stage of their disease. It is well known that parasite-induced eosinophilia is most intense during the initial infection and tissue invasion, and the level may return to normal within a few months (Lukens, 1972; Andy, 1983).

Previous studies using immunofluorescence techniques have demonstrated a high frequency of circulating anti-heart antibodies in patients with EMF (Van der Geld *et al.*, 1966; Shaper *et al.*, 1967). Similar antibodies have also been reported in patients with pulmonary tuberculosis, rheumatic heart disease and dilated cardiomyopathy (Roberts & Lessof, 1973), and are, therefore, not pathognomonic of EMF. In this study, using the indirect immunofluorescent technique, anti-heart antibodies were not demonstrated in the sera of EMF patients and controls. This finding agrees with that of Andy and Olusi (1982) who similarly found no anti-heart antibodies in fifteen Nigerians with 'early' and 'chronic' EMF.

Five (50%) of the patients in this study and four (30.7%) of the controls had immune complex levels in excess of the upper limit of normal. However, the mean concentration of immune complexes in the patients was not significantly different from the value obtained in controls. This finding most probably excludes EMF from the group of immune-complex diseases.

There are conflicting reports on serum immunoglobulin levels in EMF. Carlisle *et al.* (1972) found normal levels of IgG and IgA but slightly elevated titres of IgM in their EMF patients. However, in a recent study, Andy and Olusi (1982) found that the IgG and IgA titres in their patients with EMF were not different from those of normal controls. In our study, the mean concentrations of IgG and IgA in the EMF patients were significantly higher than those obtained in the control patients. Elevated levels of IgG and IgA have also been found in Nigerians with dilated cardiomyopathy and rheumatic heart disease (Jaiyesimi *et al.*, 1984), thus confirming that this phenomenon is not specific for EMF.

In conclusion, this study has shown that degranulated eosinophils and elevated levels of eosinophil granule basic proteins were not present in our ten cases of EMF. Eosinophilia was as common in the controls as in the patients. Similar studies are, however, required in patients with early disease in order to clarify whether these eosinophil abnormalities play a role in the pathogenesis of tropical EMF. This study has also shown no significant role for humoral immunity in the mechanism of cardiac damage in EMF.

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