

Widespread IgE-mediated hypersensitivity in Northern Sudan to the chironomid *Cladotanytarsus lewisi* ('green nimitti')

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SUMMARY

Hypersensitivity to Chironomidae (non-biting midges) has been a problem in Northern Sudan since about 1927 and is probably due to the working of dams which have produced lake-like conditions on parts of the Blue and main Niles where breeding has evidently increased. Studies were undertaken to determine whether this hypersensitivity is mediated by IgE.

Sixteen Sudanese, with bronchial asthma associated with exposure to the chironomid, *Cladotanytarsus lewisi* ('green nimitti'), were investigated. All patients gave a positive immediate-type skin reaction to an extract of the midge and the majority had markedly elevated concentrations of circulating IgE. Serum from all patients passively sensitized human lung fragments *in vitro* for the release of histamine and slow-reacting substance of anaphylaxis by the 'nimitti' antigen. This tissue-sensitizing activity could be removed by immunoabsorption with an anti-IgE. These results indicate that this widespread and important hypersensitivity in the Sudan is IgE-mediated and thus may potentially be treated by desensitization.

INTRODUCTION

Hypersensitivity to Chironomidae (non-biting midges) has been a problem in the Sudan since about 1927 (Lewis, 1956). In the affected areas *Cladotanytarsus lewisi* Freeman comprises about 80% to 90% (Lewis, 1957; Rzóśka, 1964) of small midges which have been termed 'green nimitti' (to distinguish them from an unrelated, grey, blood-sucking Simuliid nimitti which is particularly troublesome in the Dongola area). The reason for their large numbers is probably due to the working of dams which have produced lake-like conditions on parts of the Blue and main Niles where breeding has evidently increased (Lewis, 1956). The periods of midge outbreaks, which in some years reach plague proportions, are most often during the dry season when the Nile discharge is minimal (Lewis, 1957). The increase in Chironomidae has been associated with a high incidence of allergic symptoms, especially bronchial asthma and rhinitis. The exact number of individuals who are hypersensitive is uncertain, but our experience and that of others suggests that there are probably many thousands, especially in Khartoum and Wadi Halfa, where the midges sometimes appear in such vast numbers as to hinder or prevent work and recreation (Lewis, 1956). In this study we have established that chironomid-hypersensitivity is an immediate-type (type I) reaction mediated by IgE. This has an important practical implication in that it provides a rational basis for treatment by desensitization.

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MATERIALS AND METHODS

Patients. Sixteen individuals resident in the Kalakla district, 30 km south-west of Khartoum, were investigated. They all gave a clear history of bronchial asthma in association with exposure to 'green nimitti'. All were currently receiving medication with either sympathomimetics and/or disodium cromoglycate. Thirteen had a history of exercise-induced asthma. All were non-smokers. Fourteen had 'nimitti'-associated rhinitis which usually preceded the attacks of bronchospasm.

Preparation of antigen. More than 10 million midges were collected by a light-trap at Wadi Halfa and identified by Dr D. J. Lewis and Mr P. S. Cranston, Department of Entomology, British Museum (Natural History)) as *Cladotanytarsus lewisi*, less than 1% of insects belonging to a different species of Chironomid. They were dried at room temperature, defatted with ether and extracted in Coca's solution (Phillips, 1967). The material used for skin testing had a dry weight of 111 mg/ml⁻¹ and a protein content of 7.0 mg/ml⁻¹. One fortieth of this amount was used to challenge sensitized human lung fragments.

Mediator release from human lung fragments. Macroscopically normal human lung obtained at surgery, usually in association with bronchogenic carcinoma, was dissected free of pleura, bronchi and large blood vessels, sliced into fragments of approximately 50 mg and washed thoroughly in Tyrode's buffer. 200 mg of fragments were incubated with 1.8 ml of a 1:5 dilution of serum for 18 hr, washed four times with Tyrode's solution and challenged with 3.0 ml of allergen for 15 min at 37°C (Turnbull, Jones & Kay, 1976). Appropriate controls containing serum alone or antigen alone were prepared at the same time. Histamine and a slow-reacting substance of anaphylaxis (SRS-A) were measured by bioassay using the atropinised guinea-pig ileum (Brocklehurst, 1960). Residual histamine was extracted by boiling and the percentage of histamine release calculated.

Immunoglobulins and complement. IgE was removed by immunoabsorption using an unfractionated rabbit anti-human IgE (Hoechst) linked to Sepharose 4B activated by cyanogen bromide (Porath, Axén & Ernback, 1967). The monospecificity of the anti-IgE was assessed by (i) its inability to alter the levels of IgG, IgA and IgM following immunoabsorption (Table 2) and (ii) the absence of precipitin lines when tested in a single radial immunodiffusion and immunoelectrophoresis against whole human serum. IgE was measured by radioimmunoabsorption ('Phadebas', Pharmacia) and IgG, IgA, IgM, C3 and C4 by single radial immunodiffusion (Hyland). The normal serum concentrations (mean $\pm$ 2 s.d.) for IgG, IgA and IgM were obtained from a previous report (Stoop *et al.*, 1969) as were the values for IgE, C4 and C3 (Kay *et al.*, 1974). For C4 and C3, the absolute concentrations were determined from reference sera kindly supplied by Dr R.A. Thompson (Regional Immunology Laboratory, East Birmingham Hospital).

RESULTS

Fourteen of the sixteen patients had raised concentrations of total circulating IgE and in some this was markedly elevated (Fig. 1). By contrast, the levels of IgG, IgA, IgM, C3 and C4 were generally within the normal range for this laboratory. One patient had a raised IgG, one had a raised IgA and one had a

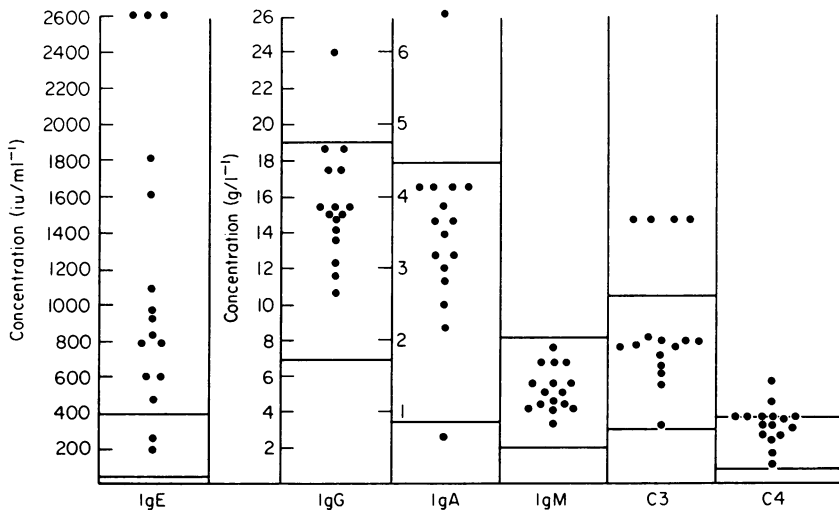


FIG. 1. Concentrations of circulating IgE, IgG, IgA and IgM and the individual complement (C) components C3 and C4 in patients with hypersensitivity to the chironomid, *Cladotanytarsus lewisi*. The normal ranges for a UK population are shown by the horizontal lines.

TABLE 1. Skin test reactivity to 'green nimitti' antigen and tissue-sensitizing capacity of sera from sixteen patients with bronchial asthma in association with hypersensitivity to the chironomid, *Cladotanytarsus lewisi*

Patient number	Age	Sex	Skin response to 'green nimitti' antigen	In vitro tissue sensitizing capacity of serum	
			Weal diameter (mm)	Histamine* (% release)	SRS-A* (μ /g)
1	23	M	7 × 7	56, 54	100, 100
2	33	F	12 × 4	21, 22	500, 550
3	37	F	12 × 8	63, 47	800, 1000
4	7	M	5 × 3	18, 24	250, 200
5	9	M	5 × 4	11, 18	100, 300
6	24	F	2 × 2	20, 29	100
7	30	F	5 × 4	26, 27	n.d.
8	25	F	2 × 2	26, 28	n.d.
9	28	F	15 × 10	43, 33	350, 450
10	25	F	10 × 8	29, 28	400, 400
11	21	M	15 × 8	22, 30	300, 300
12	17	M	4 × 4	30, 24	n.d.
13	47	M	14 × 7	30, 28	n.d.
14	56	M	20 × 20	32, 36	n.d.
15	50	F	4 × 4	17, 10	n.d.
16	35	F	10 × 10	21, 24	n.d.

* Results of duplicate assays.

n.d. = Not done.

The weal diameters were measured 20 min after the application of antigen.

Unsensitized lung fragments challenged with antigen and sensitized lung incubated with Tyrode's alone gave a histamine release of less than 5% and SRS-A in the diffusate was virtually undetectable.

TABLE 2. The capacity of IgE to sensitize lung fragments for the release of histamine and SRS-A following challenge with an extract of the chironomid, *Cladotanytarsus lewisi*

Source of serum (patient number)	IgG (g/l ⁻¹)	IgA (g/l ⁻¹)	IgM (g/l ⁻¹)	IgE (iu/ml ⁻¹)	Histamine* (% release)	SRS-A* (u/g)
Unabsorbed (1)	16.8	2.7	0.85	1344	52, 53	225, 275
Absorbed (1)	15.6	2.7	0.60	108	15, 10	10, 10
Unabsorbed (5)	16.8	2.7	1.20	4800	39, 35	700, 800
Absorbed (5)	15.6	2.8	1.20	320	17, 12	50, 50
Unabsorbed (13)	13.2	2.7	0.96	8320	51, 45	500, 500
Absorbed (13)	15.6	2.7	0.96	240	8, 17	40, 60

* Results of duplicate assays.

Controls with antibody or antigen alone gave the same results as in Table 1.

low IgA concentration, whilst in four C3 was elevated and C4 was outside the upper range in two.

All patients gave a positive skin ('prick') reaction to an extract of the allergen (Table 1). In addition, fourteen out of fifteen Sudanese without symptoms gave a positive 'prick' test to the 'nimitti' extract, whereas no reaction was elicited with this material in twenty-three grass-pollen sensitive Europeans resident in the UK. The serum from each of these individuals also passively sensitized human lung fragments for the antigen-induced release of histamine (Table 1). The diffusates from nine of the samples

were tested for SRS-A and in each this mediator was present in appreciable amounts.

Immunoabsorption was performed on three of the serum samples to remove IgE. Greater than 90% of this immunoglobulin was removed, but concentrations of IgG, IgA and IgM remained virtually unaltered (Table 2). The absorbed serum had a greatly reduced capacity for sensitizing human lung fragments for the antigen-induced release of histamine and SRS-A, thus confirming the presence of IgE antibodies directed against the 'green nimitti' antigen.

DISCUSSION

There have been numerous observations on the biology of the chironomids (Lewis, 1957). However, the origin and nature of the antigen causing hypersensitivity is not known. Chironomid hypersensitivity first became noticeable after the construction of the Sennar dam in 1925 and later a mass outbreak of the midges was reported at Wadi Halfa in association with the raising of the Aswan Reservoir (Lewis, 1956). Exploratory field trials with larvicides in Khartoum were inconclusive (Lewis, 1957) and unlikely to be of long term use in controlling the chironomids because of the prohibitive cost and unacceptable pollution. Many sufferers moved away from the Nile which caused disruption to their occupations and family life.

The normal values for immunoglobulins and complement (Table 1) were from UK Caucasians but were likely to be lower than a comparable Sudanese population. Levels of IgG, IgA and IgM were approximately 15% higher in sixty Sudanese when compared to values from UK subjects (A.R.E. Anwar & H.H. Erwa, personal communication). There was no clinical evidence to indicate that the raised concentrations of IgE were due to helminths, although this possibility cannot be excluded. The values for C3, C4, IgG and IgA, which in some were just outside the normal range, were not considered clinically significant.

The positive skin tests and the capacity of sera to sensitize lung fragments for mediator release (Table 1) and also the dependence of tissue sensitizing activity on IgE (Table 2) indicates that chironomid hypersensitivity is a classical type I reaction. The allergen did not cause histamine release from unsensitized lung fragments (Tables 1 and 2) and Europeans who have never been exposed to 'nimitti' gave negative skin reactions, indicating that mediator release is immunological rather than non-specific. It was of interest that all the Sudanese skin-tested gave a positive skin reaction to the 'nimitti' antigen irrespective of whether they suffered from allergic symptoms. If atopy is recognised as the capacity to give an IgE-mediated immediate wheal and flare reaction in the skin following the introduction of allergen, then the percentage of atopic individuals in Northern Sudan is likely to be very high. However, skin responses mediated by short-term anaphylactic IgG (Parish, 1973) may account for some of these allergen-induced reactions and this possibility is currently being investigated.

An important practical point which this study has raised is that desensitization to extracts of the midges may be of value in the treatment of this hypersensitivity, as has been shown with patients sensitive to other large multicellular organisms such as the house dust mite, *Dermatophagoides pteronyssinus* (D'Souza *et al.*, 1973).

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