

From the Department of Paediatrics, Gunma University School of Medicine,
Maebashi, Gunma, Japan.

A CASE OF MOSQUITO ALLERGY

Immunological Studies

By

S. SUZUKI, K. NEGISHI, S. TOMIZAWA, M. SHIBASAKI,
T. KUROUME & T. MATSUMURA

Allergic reactions due to insect bites continue to be a serious medical problem. A considerable amount of interesting work has been reported about hymenoptera allergy; however, there are relatively few papers dealing with mosquito allergy (1, 2, 6, 7, 11, 12, 15).

Recently, we have seen an interesting case which exhibited not only local reactions, but also various general symptoms following mosquito bites. In an attempt to clarify the immune type of response involved, the case was evaluated from both aspects of humoral and cellular hypersensitivity, and the pathogenetic mechanisms involved in this case were discussed.

CASE REPORT

History

J.I., a 14-year-old girl, was admitted to the Gunma University Hospital on November 11, 1973, because of a local skin reaction, fever and hepatosplenomegaly following mosquito bites. Her neonatal and childhood periods were complicated by a large purulent thoracic abscess and recurrent pyogenic infections at BCG injection sites. Consequently, she did not receive additional BCG injections. The other ordinary childhood immunizations were successfully tolerated. In the family history, her mother was sensitive to lacquer, but she had no history of atopic tendency. Mosquitoes had never caused the girl any unusual discomfort until she was 3 years old when she experienced marked local swelling and pain following bites. Seven years later, mosquito bites resulted in even greater pain and much larger swelling accompanied by high fever and regional adenopathy.

The local reaction showed great variety, from the early reaction which appeared immediately after the bite to the delayed one which occurred 1 or 2 days later. The first manifestation was an inflammatory reddish swelling, similar to the usual positive immediate skin reaction. In the course of about 4 h, a vesicle formed at the centre, gradually changed to a purpuric tone simulating the Arthus reaction, and finally formed a crust. The crust usually peeled off about 2 months later leaving a deep ulceration. When the redness appeared immediately after the bite, the reaction quickly disappeared without any general adverse reactions; but a more delayed reaction was accompanied by high fever, general malaise and regional adenopathy which persisted for some days. These reactions had gradually lessened after reaching a peak when the child was 11 years old; the new signs such as hepatosplenomegaly and tachycardia developed later.

Physical Examination

On admission, physical examination revealed a girl with several excavated scars of about 1 cm in diameter on the face and legs. The outlines of the liver and spleen were palpable 3 and 5 f.b., respectively, below the costal margin; lymphnodes were slightly enlarged in all areas (Fig. 1). The temperature was 36.1° C, pulse 120/min, and blood pressure 110/80.

Laboratory Findings

Blood examination disclosed a moderate pancytopenia, but a bone marrow aspirate showed normal cellularity. The absolute lymphocyte count was 740/ μ l and the total T-cell population detected by modification of the method of Wybran et al. (17) was 52 per cent. Thrombocytes were greatly reduced in number, being 10 800/ μ l, but bleeding and coagulation times were within normal limits. Other abnormal laboratory findings were as follows: CCF (cephaline-cholesterol flocculation test) (Hanger) (+++); ICG (indocyanine green test) 3 per cent; alkaline phosphatase 96 units (14–80 units); ASLO (antistreptolysin-O) 833 Todd units (< 250 TU); RA (rheumatoid arthritis factor) (+++); serum iron 41 mg/100 ml. Serum protein electrophoresis exhibited hypergammaglobulinaemia, and the quantitative assay of immunoglobulin resulted in a marked increment of IgA (1250 mg/100 ml) and IgE (over 5300 IU) (Table 1). Electrocardiogram findings included tachycardia and a prolonged PQ interval (0.2"), occasionally showing an A-V block of the Wenckebach type.

Histological Skin Findings

A skin biopsy of the 2-month-old scar following a mosquito bite showed non-specific inflammatory reaction, i.e. a moderate thickened, fibrous corium, and necrosis of adipose tissue. The vessels extending from the corium to the adipose tissue layer exhibited a proliferation of endothelial cells and extravasation of red cells but no fibrinoid degeneration. In the specimen of cervical lymphnode,

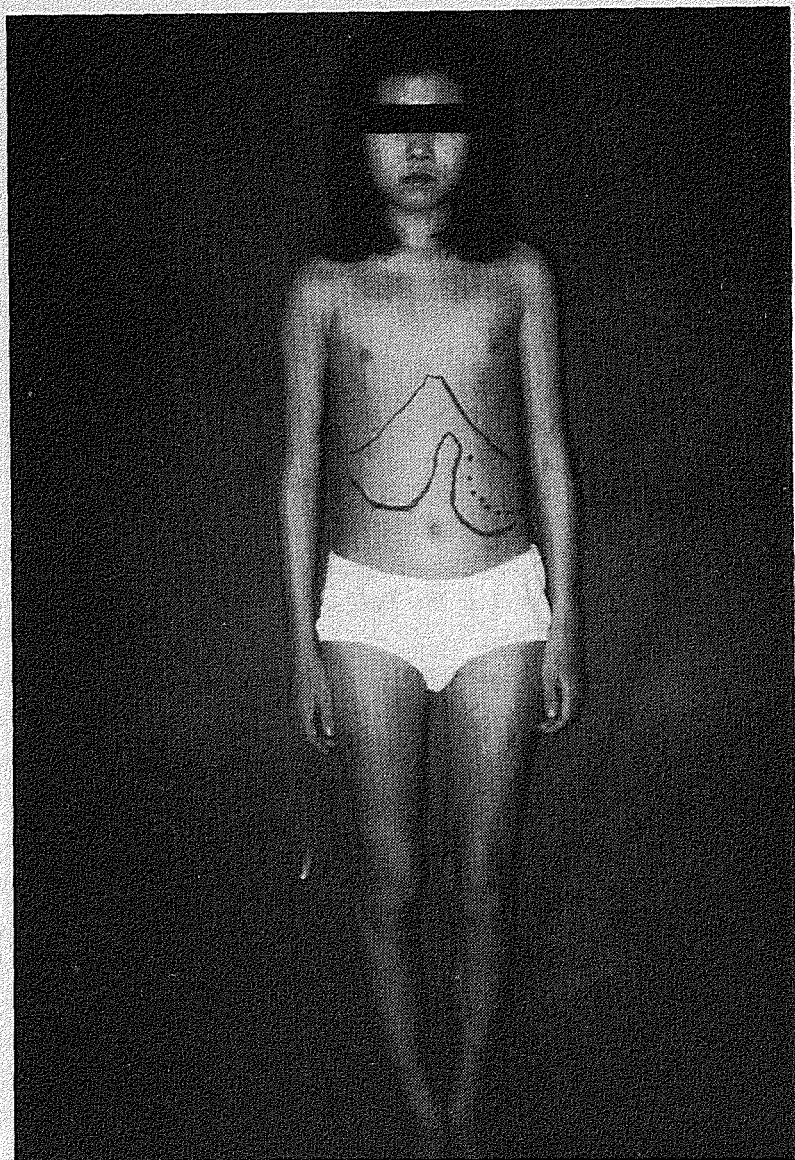


Fig. 1.

Case: J.I., a 14-year-old girl. Note the hepatosplenomegaly and the excavated scars on the legs.

generally reduced lymphocytes, ill-demarcated follicular architecture of cortex, and a proliferation of RES or plasma cells were detected.

MATERIAL AND METHODS

Antigens

Three mosquito antigens (*Aedes vexans nipponii*, *Culex tritaeniorhynchus*, and *Anopheles sinensis*), and other inhalant allergens were obtained from Torii

Drug Company (Tokyo, Japan). The other antigens used in the intracutaneous skin tests and the *in vitro* ^3H -thymidine (^3H -TdR) incorporation tests were the following: Bacto-phytohaemagglutinin-P (PHA-P) (Difco Laboratories Inc., Detroit, Michigan), purified protein derivative (PPD) (Japan BCG Company, Tokyo, Japan), streptokinase-streptodornase (SK-SD) (Varidase, Lederle, Pearl River, New York) and human serum albumin (HSA) (Sigma Chemical Company, Fraction V).

TABLE 1
Immunological Laboratory Findings.

Total protein	8.7 g/100 ml	
Albumin	44.9 %	
α_1 -globulin	3.6	
α_2 -globulin	5.5	
β -globulin	12.0	
γ -globulin	33.0	
Immunoglobulin		
IgG	1950 mg/100 ml	
IgA	1250	
IgM	150	
IgE	5300 IU	
T rosette-forming cell	52 %	
	Delayed skin reaction (48 h)	^3H -TdR incorporation
PHA-P (1 μg)	$\frac{0 \times 0}{9 \times 10}$	294664 ct/min (429)*
PPD (0.05 μg)	$\frac{0 \times 0}{0 \times 0}$	7548 (19)
SK-SD (1 μg)	$\frac{11 \times 11}{22 \times 22}$	ND**
Candida (1:1000)	$\frac{0 \times 0}{15 \times 18}$	ND

* Stimulation index.

** Not done.

Skin Tests

Prick tests were done by the conventional procedure, using 1:20 solution of a series of the above-mentioned antigens.

Intradermal skin tests were performed on the volar aspect of the forearm or the back using a threshold dilution technique. One-fifth ml of a ten-fold dilution of mosquito antigens were injected intradermally, starting with a low concen-

trated solution of $1:10^{-7}$ (0.102 PNU/ml) to prevent adverse reaction. After 15 min, the reactions were graded and those reactions in which the mean diameters of weal and erythema were greater than 6 and 11 mm, respectively, were considered as positive. If negative, more concentrated solutions were tried until a good positive reaction was obtained and the least dilution required for a positive reaction was considered as the end point. The delayed reaction was observed again after 48 h.

Prausnitz-Küstner (P-K) reactions were performed, using 0.1 ml each of patient serum screened for Australia antigen and Wasserman reaction. Forty-eight hours later each injection site was challenged with 0.02 ml of a 1:1000 mosquito antigen. As controls, heat-inactivated serum (56° C, for 2 h) and saline were used.

Specific IgE Antibody (RAST)

Specific IgE antibodies against mosquito antigens were determined by the method of Ceska (4), using paper discs coupled with the ratio of 20 pieces of disc to an antigen solution of 5 mg protein concentration/ml. The discs of food allergens and ^{125} I-labelled anti-IgE serum were obtained from RAST kits (Pharmacia, Uppsala, Sweden). Details of the procedure have been described in a previous paper (9).

Neutralization Test by RAST

Each 50 μ l of sera was mixed with a double volume of buffered saline or antigen solution (1:20) and incubated for 2 h at room temperature, after which the RAST were determined.

Double Immunodiffusion in Agar

Double immunodiffusion was carried out by the method of Ouchterlony (13) using 1 per cent agarose (Behringwerke, Germany). The antigen wells containing 5 mg/ml were set up against patient sera. After incubation for 48 h in a cold room, the agar plate was gently washed with buffered saline (pH 7.6) and anti-IgG, anti-IgM, anti-IgA rabbit sera (Hyland, California) were applied in the serum wells.

Fluorescent Antibody Studies on Skin Section

After fixing in acetone for 10 min, the specimens were stained with the anti-immunoglobulin sera commercially conjugated with fluorescein-isothiocyanate (FITC), and washed. They were then mounted in the PBS-buffered glycerine and examined under a fluorescent microscope. Control specimens were stained with a non-specific rabbit globulin conjugated with FITC.

In vitro Transformation Test

The incorporation of ^3H -TdR into newly formed DNA was used as an index of lymphocyte proliferation. The general method has been described in an earlier publication (8).

RESULTS

The prick tests with three mosquito antigens and 24 inhalant allergens were negative, except for the three mosquito antigens. Intracutaneous tests against the three mosquito antigens also showed a strong immediate reaction but no Arthus and delayed-type reaction. The thresholds of intracutaneous tests were $1:10^5$, $1:10^5$ and $1:10^6$ against *A. vexans nipponii*, *C. tritaeniorhynchus*, and *A. sinensis*, respectively (Table 2).

TABLE 2

Threshold of Intracutaneous Test, P-K Titre and RAST Counts against Mosquito Antigens.

Antigens	Threshold of intracutaneous test	P-K test	RAST counts
<i>Aedes vexans nipponii</i>	$1:10^5$	1:640	1736 ct/min
<i>Culex tritaeniorhynchus</i>	$1:10^5$	1:160	1618
<i>Anopheles sinensis</i>	$1:10^6$	1:320	910
Control (HSA)			269

P-K tests were successfully accomplished in two recipients. The highest P-K titer was 1:640 of *A. vexans nipponii*, followed by *A. sinensis* and *C. tritaeniorhynchus*, showing 1:320 and 1:160, respectively. No P-K reaction was observed with the heated patient serum (Fig. 2), (Table 2).

Although there was some paradoxical relation between the thresholds of intracutaneous tests and the RAST counts, the presence of specific IgE antibodies against mosquito antigens was also confirmed by RAST technique. That is, the RAST counts against the three mosquito antigens were 1736, 1618 and 910 ct/min, respectively, while that against HSA, to

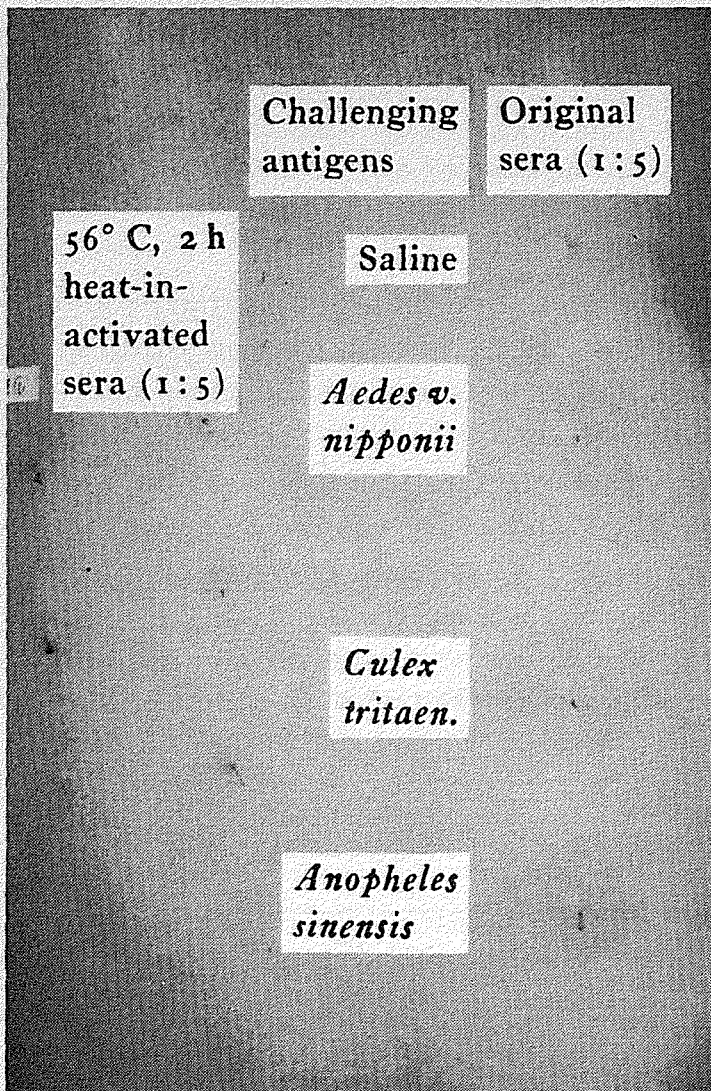


Fig. 2.

The P-K reactions were definitely elicited at the sites prepared with the original sera, while no reaction was observed at the site challenged by saline or the sites prepared with the heat-inactivated sera (56° C, 2 h).

which humans would not be sensitized, was 269 ct/min (Table 2). Further evidence supporting the antigen specificity is that the patient had low RAST counts against indifferent antigens such as egg and milk, and the other cases with high serum IgE comparable to the patient showed relatively low RAST counts against mosquito antigens (Table 3). The neutralization tests applied to the RAST technique resulted in a 52 per cent,

TABLE 3
RAST Counts against Mosquito and Food Antigens.

Name	Disease	Total IgE (IU)	RAST counts (ct/min)			
			Mosquitoes		Foods	
			<i>A. vexans</i> <i>nipponii</i>	<i>C. tritaeni-</i> <i>orhynchus</i>	<i>Anopheles</i> <i>sinensis</i>	Milk Egg
J.I.	Mosquito allergy	5300	1736	1618	910	942 416
J.I.'s mother	Healthy	430	608	431	477	
Y.W.	Mosquito allergy	450	1780	786	595	
K.K.	Nephrotic syndrome and mosquito allergy	50	597	669	610	
M.N.	Bronchial asthma	1700	533	844	808	632 4013
N.K.	Atopic eczema	7600	672	645	722	5066 5539
S.W.	Purpura	4000	281	464	318	
K.N.	Healthy		423	447	587	

48 per cent and 33 per cent reduction of RAST counts after absorption with *A. vexans nipponii*, *C. tritaeniorhynchus* and *A. sinensis*, respectively. However, the fact that to some extent, one antigen absorbs the RAST counts more than another, seems to suggest the presence of cross-antigenicity between them (Table 4).

TABLE 4

Neutralization Test of IgE Antibody against Mosquito Antigens by RAST.

	Buffer solution	Neutralizing antigens (1:20)		
		<i>A. vexans nipponii</i>	<i>C. tritaeniorhynchus</i>	<i>Anopheles sinensis</i>
<i>A. vexans nipponii</i>	1658*	796 (-52%)	1108 (-33%)	1256 (-24%)
<i>C. tritaeniorhynchus</i>	1543	1128 (-27%)	798 (-48%)	1262 (-18%)
<i>Anopheles sinensis</i>	872	659 (-24%)	671 (-23%)	584 (-33%)

* Count per minute (ct/min).

The precipitating antibody against the three mosquito antigens could not be detected in the patients sera by the double immunodiffusion test. The immunofluorescent antibody technique also revealed absence of deposits of IgG, IgM, IgA, IgE, β_1 C or fibrinogen around the vessels.

The incorporation of ^3H -TdR into lymphocytes stimulated with *A. sinensis* was measured in six cases, comprising the patient, her mother, and four age-matched controls. Using 1:100 antigen solution, all cases responded maximally to the antigen after a 4-day incubation period, showing from 600 to 1700 ct/min (Fig. 3). On the other hand, by changing the antigen concentration from 1:20 to 1:2500 for a 4-day incubation period, the peak response was observed around 1:500 (Fig. 4). Parallel to her mother's data, the patient responded maximally with smaller doses than that of the four controls; but to

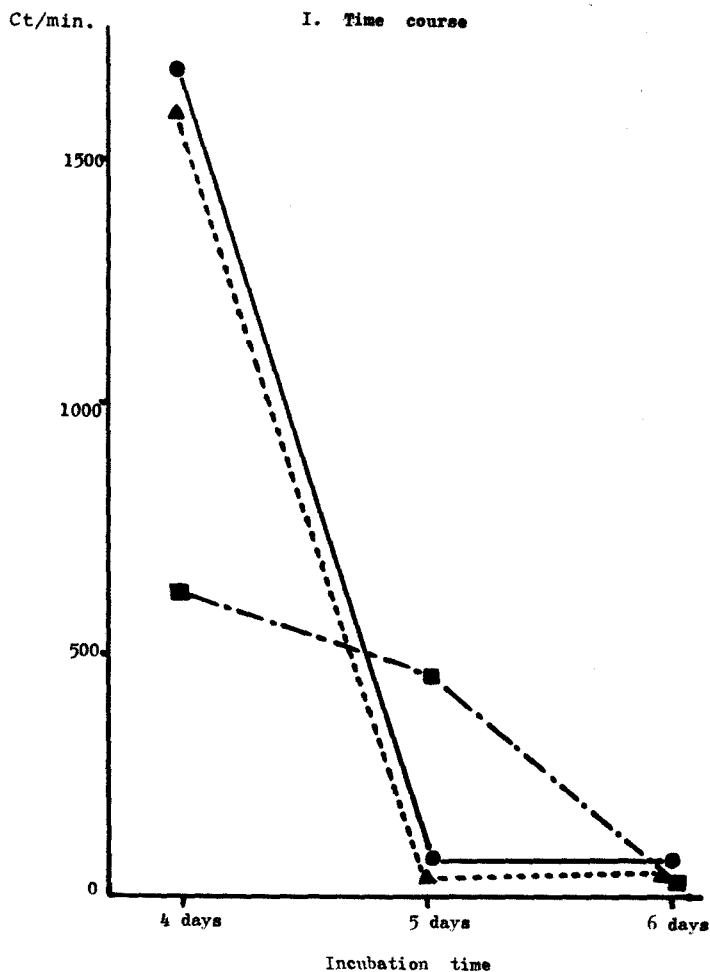


Fig. 3.

Comparison of kinetics of incorporation of ^3H -TdR by the lymphocytes from the patient (●—●), her mother (▲---▲) and a healthy person (■—■). The cultures were incubated for 4, 5 or 6 days with 0.1 ml of 1:100 antigen solution of *Anopheles sinensis* and exposed to 1 μCi ^3H -TdR for the final 16 h. The mean ct/min of duplicate culture is shown.

reach a final conclusion on this point, further experiments will have to be done with a larger number of cases. To evaluate other cellular immunity of this patient, skin tests against PHA-P, PPD, SK-SD and *Candida albicans* were performed. The patient failed to show a delayed response to PPD while she responded to the other three antigens (Table 1).

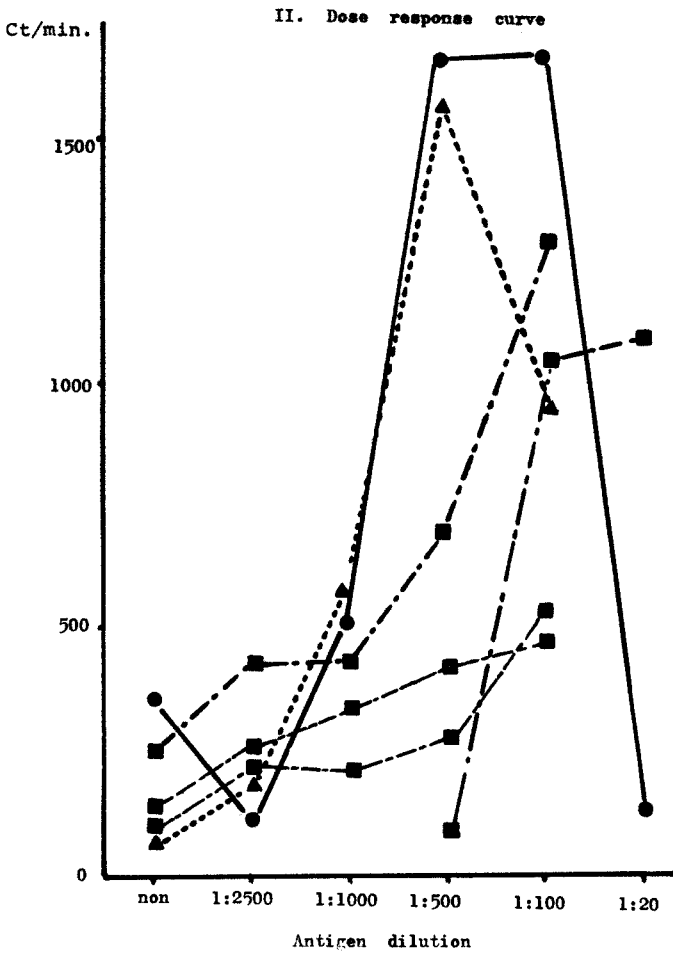


Fig. 4.

Comparison of kinetics of incorporation of ^3H -TdR by the lymphocytes from the patient (●—●), her mother (▲---▲) and healthy persons (■---■). The cultures were incubated for 4 days with various doses of antigen solutions of *Anopheles sinensis* and exposed to $1\ \mu\text{Ci}$ ^3H -TdR for the final 16 h.

The mean ct/min of duplicate culture is shown.

DISCUSSION

In an excellent review of mosquito allergy, Heilesen (6) has pointed out that either an immediate or a delayed reaction develops following mosquito bites. Occasionally, the latter type of reaction may assume the Arthus character with necrosis. In our case, it seems evident that type I, type III and type IV hypersensitivities (5) are involved in the reactions caused by mosquito bites. As mentioned above, we confirmed

the presence of a type I reaction by means of the intracutaneous test, P-K test, and RAST, but we could not detect any precipitating antibody with either the double immunodiffusion or immunofluorescent antibody techniques. As regards the type IV reaction, skin tests and ^3H -TdR incorporation tests were made. The skin test failed to show a positive delayed reaction, while the lymphocytes of the patient were definitely stimulated by the mosquito antigens. Similar discordant phenomena have been demonstrated in pollen allergy by several authors in the last few years (3, 10, 14, 15, 18). The significance of *in vitro* lymphocyte activation by allergens in relation to the triggering mechanisms of atopic disease was not fully elucidated. Our data are somewhat different from that for pollen (15) in which only the lymphocytes from untreated, atopic individuals respond to the pollen extract, whilst those from hyposensitized atopic or non-atopic persons do not. Of course, the discrepancy is not absolute, and it may be due to the difference in antigens or their optimal doses.

Another important question is why this patient developed irreversible and deleterious general reactions, just with mosquito bites. The patient's mother, who had no significant reaginic antibody against mosquito antigens although her lymphocytes were stimulated with smaller doses than either the controls' or the patient's, did not experience any adverse reaction from mosquito bite. The fact indicates that both the disturbances of the reaginic antibody and lymphocyte response play important roles in the pathogenesis. In addition, the abnormal reaction to BCG, the deficit of the delayed reaction against PPD, pathological findings of lymphnode and moderately reduced T-cell population lead us to suspect the presence of some abnormality of cellular immunity in the individual. There is also the possibility that a toxic substance which is different from the allergenic one may be responsible for such an adverse reaction.

SUMMARY

A girl aged 14 years, who showed local skin lesions and generalized reactions such as fever, hepatosplenomegaly, hypergammaglobulinaemia and pancytopenia following mosquito bites, has been evaluated with various immunological methods. The intracutaneous tests, P-K tests and RAST confirmed the presence of specific IgE antibody against mosquito antigens, but no precipitating antibody was detected by either the double immunodiffusion or immunofluorescent antibody techniques. As far as the type IV reaction is concerned, the skin test failed to show a positive delayed skin reaction, but the lymphocyte incorporation of ^3H -TdR exhibited a maximal response with smaller doses than the controls.

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Authors' address:

Shigeyoshi Suzuki, M.D.
 Department of Pediatrics
 Gunma University School of Medicine
 Maebashi, Gunma
 Japan

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