HUMAN NATURAL ANTIBODIES TO *CULEX QUINQUEFASCIATUS*: AGE-DEPENDENT OCCURRENCE

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ABSTRACT. The titer of anti-*Culex quinquefasciatus* antibodies which were mostly of IgG and IgE isotypes was determined in humans living in *Wuchereria bancrofti* endemic regions. A progressive increase in the titer of antibodies was observed with age. In contrast, sera from people living in regions where the *Cx. quinquefasciatus* is of low prevalence were found to have markedly reduced IgG and almost zero IgE levels.

Vertebrates elicit specific immunological responses induced by the bites of blood-sucking insects (Nelson et al. 1977). Such reactions in man caused by mosquitoes are quite prevalent, commonly characterized by local cutaneous hypersensitivity. Precipitating antibodies against *Aedes communis* (De Geer) have been demonstrated in human sera from Finland (Ailus et al. 1985). We have described the presence of antibodies to *Culex quinquefasciatus* Say, a vector of the filarial parasite *Wuchereria bancrofti*, in normal and filarial infected persons in Orissa, India (Das and Dash 1986). The antibody level in the 2 groups did not differ significantly. In the present report an attempt has been made to determine the age-specific prevalence of anti-*Culex* IgG and IgE isotypes in the healthy human population.

Rearing of *Cx. quinquefasciatus* in the laboratory was carried out as described earlier (Das and Dash 1986). Briefly, mosquitoes were collected from the field and reared at 25°C, 70% RH. Gravid females were kept in an insect cage containing ovitraps for egg laying, and the eggs collected were transferred to water trays. Emerged adult mosquitoes were maintained on a diet of 10% glucose solution.

Female mosquitoes (approximately 50) were collected in test tubes and immobilized by exposure to −20°C for a few seconds. Salivary glands of the mosquitoes, isolated by dissection under microscope, were sonicated for 10 min in phosphate buffered saline (PBS) (Branson sonifier 450, 30% duty cycle, output control = 3) and cold centrifuged at 8,800 × g (Remi C-24 centrifuge). The clear supernatant was removed, analyzed for protein content with Folin reagent and stored at −20°C.

Human sera (n = 75) were obtained from normal, healthy individuals from Puri district of Orissa, India, a region endemic for *W. bancrofti* filariasis (Das et al. 1988), where the prevalence of *Cx. quinquefasciatus* was found to be 77%. Sera (n = 19) were also collected from individuals (9 children and 10 adults) living in a region (Anugul, Orissa, India), where *Cx. quinquefasciatus* constituted only 5% of the total mosquitoes present, anopheline mosquitoes being the major species (>75%) found. Sera of children from this region were used as the (negative) control. Both the control and experimental group live under similar socioeconomic conditions in rural villages. The ELISA plate wells were coated with *Culex* antigen from salivary glands (100 µl, 2 µg/ml) in 0.05 M carbonate buffer pH 9.5. Bovine serum albumin (BSA) was used (0.4%) to prevent nonspecific binding. Plates were washed with PBS containing 0.01% Tween-20 followed by the addition of human sera with a doubling dilution (from 1/75 dilution onward). Each dilution was tested in duplicate. Plates were incubated for 3 h at 37°C and then washed again. Peroxidase-conjugated (100 µl) rabbit anti-human IgG and IgE, diluted 1/1,000 in PBS-Tween, were added and plates were incubated overnight at 4°C. The plates were then developed with freshly prepared substrate solution (o-phenylenediamine containing H_2O_2). Intensity of the color developed by adding 4 N H_2SO_4 was read (absorbance at 492 nm) by an automatic ELISA reader (Bio-Rad model 2550). It was observed that children's (n = 9) control sera at 1/75 dilution itself exhibited a zero absorbance value for IgE and low values for IgG. The mean IgG value was 0.03 ± 0.01; titer was expressed as the arithmetic mean ± 1 SD. The last dilution exhibiting color (absorbance > 0.07) was considered as the titer. This value also exceeded the above mean + 3 SD of control (children) sera.

It was established in the initial screening that IgM antibodies to *Culex* antigens were of negligible proportions in comparison with IgG and IgE isotypes. The titer of these later antibodies was therefore determined (Table 1). The sera from 75 people were arranged in 5 groups for comparison—group A for children (<5 years), group B (10–16 years), group C (18–40 years),

Table 1. Titer* of *Culex quinquefasciatus* specific antibodies in human sera.

Group	Age (yr) (mean ± SD)	*n*	Titer (range) IgG	Titer (range) IgE
A	1–5 (4.1 ± 1.2)	10	337 ± 155 (75–600)	180 ± 133 (75–300)
B	10–16 (12.9 ± 1.5)	21	1,109 ± 660[a] (300–2,400)	300 ± 272 (75–600)
C	18–40 (29.4 ± 5.9)	21	1,500 ± 620[a] (1,200 ± 2,400)	850 ± 354[a] (300–2,400)
D	41–50 (43.5 ± 4.0)	13	1,610 ± 633[a] (900–2,400)	790 ± 308[a] (300–1,200)
E	51–70 (61.4 ± 5.5)	10	1,170 ± 457[a] (900–2,400)	810 ± 170[a] (600–1,200)

* Titer expressed as arithmetic mean ± 1 SD.
[a] Compared to Group A, $P < 0.01$.

group D (41–50 years) and group E (51–70 years). From Table 1 it is clear that there was a progressive increase in the titer of antibodies with increase in age. The titer of IgG antibodies (but not of IgE) increased considerably in group B (mean age 12.9 years) compared with that in group A (mean age 4.1 years). This increase in IgG titer continued, albeit marginally, in group C and D (mean age 29.4 and 43.5 years, respectively). Whereas in group E (61.4 years) there was a decrease in the titer, which was however not statistically significant ($P > 0.05$) in comparison with group D. A reduction in antibody response especially of IgG to foreign antigens is known to be associated with aging (Walford 1969). In contrast to IgG, IgE titer did not differ significantly between group A and B, but it showed considerable enhancement in group C and others. It can be suggested that the maturation of IgE titer occurred at a later age at group C (29.4 years), whereas IgG titer attained a maximum earlier. Comparison of anti-culex antibody levels in the control and study population may be made. The mean absorbance values for IgG-ELISA in the children's group of control ($n = 9$, 1–13 years, mean 6.5 ± 2.8 years) and experimental ($n = 10$, 1–5 years, mean 4.1 ± 1.1 years) sera at 1/75 dilution are 0.03 ± 0.01 and 0.28 ± 0.10, respectively; IgE levels are zero in the former. Even the adults' control sera ($n = 10$, 24–50 years, mean 30.1 ± 9.9 years) have a lower IgG titer of 300 and an almost negligible IgE titer. This is in marked contrast to the experimental group as noted above.

Increase in the titer of antibodies with age may reflect an increase in exposure to *Culex* bites. The *Culex* antibodies were detected in the sera of people living in highly endemic regions of filariasis. The biological role of such antibodies remains unknown at present. However, some possibilities may be suggested. It is known that animals become immunologically sensitized after being fed on by hematophagous arthropods. This immunological response produced resistance to further feeding. For example, repeated feeding by *Rhipicephalus appendiculatus* on rabbits produced a resistance mediated by specific antibodies to further tick feeding (Shapiro et al. 1987). Similarly, cattle vaccinated with antigenic preparation from female ticks, *Boophilus microplus*, exhibited a higher degree of immunity to the parasite (Willadsen et al. 1988). It has been postulated that the immunological response induced by blood-sucking insects perhaps imparts the host with protection from diseases by interfering with pathogen transmission (Brown 1988). It would be an interesting possibility if the antibodies to *Cx. quinquefasciatus* that are acquired naturally in man may slow the transmission of filariasis by affecting *Culex* ecology. The control and experimental sera were obtained from regions that differ in the prevalence of *Cx. quinquefasciatus*. The presence of markedly reduced antibody level in the control population, although the number of samples is not high, was interesting since these people are normally exposed to the bites of other mosquitoes, especially the anophelines. It probably suggests that naturally occurring anti-culex antibodies in man are not highly cross-reactive. The quantitation of anti-culex antibody level in man could prove to be useful as a measure of exposure to mosquito bites in field studies.

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