Immunoglobulin Response in Malaria in Ethiopia: Association of Splenomegaly with Increased Concentration of IgM[1] (36857)

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The sera of many Africans contain increased concentrations of gamma globulins (1) and immunoglobulins (2–5). In some cases, the increased globulin concentrations may be due to chronic exposure to malaria (1, 6–8). In volunteers, malarial infection causes an increase in the concentrations of the major classes of serum immunoglobulins (9) and is associated with the appearance of malarial antibody (10–12).

The present report contains results of IgG, IgA and IgM analyses on sera of 57 patients from Ethiopia, each of whom was examined individually. We found a significant association between the presence of splenomegaly and of increased concentrations of IgM in the serum.

Materials and Methods. I. Patient selection and examination. The 57 patients from Ethiopia were divided into 3 groups. Group I (39 patients) included persons who resided near Ebnat, a village in a malarious region about 200 km east of Gondar. The lowest elevation in the Ebnat region is 1780 meters and the highest, 2220 meters; seasonal outbreaks of malaria occur, usually in December. The remaining 18 patients lived above 2000 m in nonmalarious regions of Ethiopia and were studied while in the hospital in Gondar. Ten of the latter 18 patients had a history of malarial illness during prior residence in a malarious region (Group II) whereas 8 of the patients had neither a history of malaria nor had lived in a malarious region (Group III). A complete history and physical examination was performed on each patient.

II. Specimen procurement. After alcohol preparation of the skin, about 8 ml of blood was taken from the antecubital vein into a sterile vacuum tube. Blood specimens from patients in Gondar were processed without delay. The specimens taken from patients in Ebnat village were kept unrefrigerated, at a maximum temperature of about 25° for 3 days during transport to Gondar where the serum was separated by centrifugation, frozen and kept at −20°. Specimens were then shipped by air to Houston where they were kept at −70° until assayed for immunoglobulin content 6 mo later. Thick and thin blood smears for malarial parasites were made on acid-cleaned microscope slides at the time of each venipuncture. The slides were processed with Giemsa's stain. For control purposes, we obtained serum specimens from 72 normal persons in Houston, TX, matched for age and sex with the Ethiopian patients.

III. Experimental procedures. Concentrations of IgM, IgA and IgG were measured by single radial diffusion in agar using commercially prepared plates (Hyland Laboratories, Los Angeles, CA) and procedures described previously (13). Sucrose density gradient ultracentrifugation was carried out using 5–20% gradients (13)

Results. I. Clinical findings. Of the 39 patients of Group I residing in malarious regions, 19 (49%) had hepatomegaly, 27 (69%) had splenomegaly, and 20 (51%) had malarial parasites on blood smears (10 falciparum, 9 vivax and 1 mixed infection). All 39 patients gave a history of malaria; in 24 patients (62%) the symptoms had occurred

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TABLE I. Immunoglobulin Concentrations in Malarious and Nonmalarious Regions of Ethiopia.

	No. of cases	Serum immunoglobulin concn[a] (mg/ml)					
		IgG		IgA		IgM	
		Mean	Range	Mean	Range	Mean	Range
Malarious region (Group I)	39	23.0 (±8.8)[b]	13.0–65.0	2.9 (±1.4)	0.9–7.5	3.3 (±3.1)	0.6–11.7
Nonmalarious region							
Prior exposure to malarious regions (Group II)	10	17.5 (±4.8)	12.0–30.0	3.3 (±0.9)	1.8–4.3	1.7 (±0.7)	0.9– 3.3
No prior exposure to malarious regions (Group III)	8	14.4 (±4.3)	8.5–21.0	2.4 (±1.1)	1.0–4.5	1.0 (±0.8)	0.5– 2.8
Control group (Houston)	72	15.0 (±6.5)	7.2–29.1	1.7 (±0.8)	0.7–6.0	1.0 (±0.5)	0.3– 2.0

[a] The following differences between means were significant at the .01 level: (1) IgG, Group I vs Group III, Group I vs control group. (2) IgA, Group I vs control group, Group II vs control group, (3) IgM, Group I vs control group, Group II vs control group, Group I vs Group III.

[b] ± One standard deviation.

within the preceding 3 weeks, in the remaining 15 patients, symptoms were more remote. In contrast, acute malarial symptoms, hepatomegaly, and detectable parasitemia were not seen in patients in Groups II and III residing in the nonmalarious regions. However, 6 (60%) of the patients in Group II had splenomegaly resulting from the previous exposure to malarious regions.

II. Serum immunoglobulin analyses. A. Serum immunoglobulin concentrations. The mean IgG concentration of Ethiopian patients living in the malarious area (Group I) was significantly higher than the mean of persons in Group III who had not been exposed to malaria (Table I). The distribution of IgG concentrations among the individuals in Group III was similar to that of the Houston controls.

The mean concentrations of IgM followed a similar pattern. About one-third of the patients living in the malarious region had IgM concentrations exceeding 3.4 mg/ml which was the highest value seen in any of the other patients or in the controls. On the other hand the IgA concentrations were approximately the same in all 3 Ethiopian groups, the mean IgA concentrations being slightly higher than those of the Houston group (Table I).

The presence of low molecular weight components of serum immunoglobulins which would cause abnormally high readings in the immunodiffusion tests was ruled out by determining the sedimentation coefficients of immunoglobulins in selected sera. The mean sedimentation coefficient of IgM in the 8 sera with the highest IgM concentrations was 18.15 (± 1.1 SD). IgM sedimented as a single peak in all of the sera. The mean sedimentation value of IgG in the 15 sera with the highest IgG concentrations was 6.9S (± 0.4 SD). The sedimentation value of IgA in the same 15 sera was 6.9S (± 0.5 SD).

B. Relationship of symptoms and parasitemia to immunoglobulin concentration. The mean IgG, IgA and IgM concentrations in the 24 patients with acute malarial symptoms compared with the 15 with remote symptoms were not significantly different. However, the mean serum immunoglobulin concentrations of patients with *P. vivax* parasitemia (IgG = 26.4 mg/ml, IgA = 3.6 mg/ml and IgM = 4.6 mg/ml) tended to be higher than those of patients with *P. falciparum* parasitemia (IgG = 21.7 mg/ml, IgA = 2.0 mg/ml and IgM = 2.0 mg/ml) and those without detectable parasitemia (IgG = 19.5 mg/ml, IgA = 2.0 mg/ml and IgM = 2.5 mg/ml) ($p < .05$).

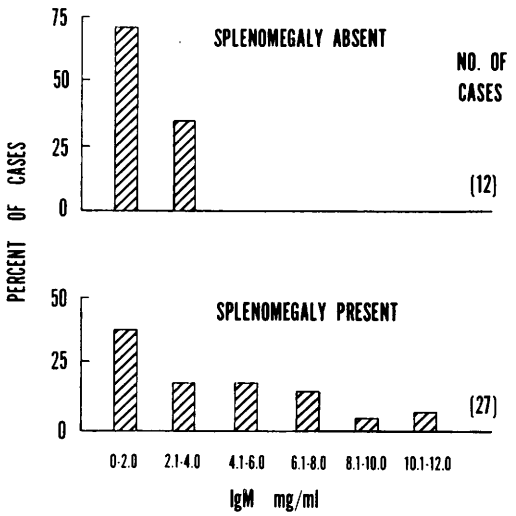

FIG. 1. Association of splenomegaly and increased IgM concentrations in serum.

C. Relationship of spleen size and immunoglobulin concentration. The mean concentrations and distribution patterns of IgG and IgA in patients with splenomegaly were similar to those of patients without splenomegaly. In contrast, the mean IgM concentration of the 27 patients with splenomegaly was significantly higher than that of the 12 patients without splenomegaly (4.0 mg/ml vs 1.6 mg/ml), due to the large number of individuals with IgM concentrations above 3.4 mg/ml (Fig. 1). Among the 27 patients with splenomegaly, mean IgM concentrations were identical in those with acute and remote symptoms.

Discussion. The present studies demonstrate that Ethiopian patients living in malarious areas have increases in the 3 major classes of immunoglobulins. Occasional patients have remarkably high concentrations of individual immunoglobulins, as high as 4 times the normal mean for IgG and IgA, and nearly 12 times the normal mean for IgM.

Of particular interest were the findings of essentially normal serum immunoglobulin concentrations in patients from Ethiopia who had always resided in nonmalarious areas and of slightly higher-than-normal values in patients residing in the same area, but who had lived previously in malarious regions. These findings suggest that the stimuli causing the increased immunoglobulin concentrations exist only in the malarious regions, but that even after leaving these regions, an individual retains evidence of a residual stimulatory effect. This observation is similar to results of a study by Schofield (14) who reported that the gamma globulin concentrations in the sera of West Africans living in Great Britain for more than 6 yr were slightly higher than those of Europeans. Whether antigenic material persists in the body, or whether the effect is due to stimulation by cross-reacting antigens or to other causes cannot be determined from the present data.

Splenomegaly warrants a high degree of suspicion of malaria and is commonly used as a means of estimating the prevalence of malarial infection (15). It was interesting that the Ethiopian patients who had splenomegaly exhibited higher IgM concentrations than the remaining patients without splenomegaly. Turner and Voller (16) reported a trend toward increased IgM levels in Nigerians with parasitemia, but the authors did not correlate their findings with spleen size. Likewise, measurement of splenic enlargement in adults was not carried out in a recent study from Gambia, in which IgM concentrations were noted to be elevated in some but not all patients (17).

Splenomegaly with increased IgM levels in the tropics has previously been described with visceral leishmaniasis (18), schistosomiasis (19), and trypansomiasis (18, 20). The persistent elevation of IgM in trypanosomiasis is thought to be due to antigenic variation of *T. gambiense* (20). Whether similar or other mechanisms are responsible in malaria for the association observed in the present studies of splenomegaly and increased IgM concentration requires future investigation.

Summary. Persons living in malarious regions of Ethiopia had significantly higher serum concentrations of IgG and IgM but not of IgA than those Ethiopians who had never been exposed to malarious regions. Immunoglobulin concentrations of the latter group were quite comparable to those of normal controls from Houston. Splenomegaly in patients with malaria was associated with

markedly increased concentrations of IgM in the serum.

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