Distribution of Malarial Antibody in Maternal and Cord Sera

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There is evidence from previous studies that the raised γ -globulin level observed in tropical populations is closely associated with malarial infections (Cohen, McGregor, and Carrington, 1961; Kuvín *et al.*, 1962; Edozien, Gilles, and Udeozo, 1962; Cohen and McGregor, 1963). Though the malarial antibody activity was thought to be mostly of immunoglobulin G (IgG), experimental proof of this was not revealed until Abele *et al.* (1965) fractionated, by means of Sephadex G-200, the sera of Caucasian volunteers who were experimentally infected with *Plasmodium vivax*, and identified the various effluent fractions by the indirect fluorescent antibody technique. Though the immune response in these volunteers was mainly of a primary nature, it should be emphasized that this does not fully reflect the type of immune response that may occur in humans who are chronically exposed to malarial infection. Hence the distribution of malarial antibodies in sera of persons chronically infected with malaria might be different from those who have a primary infection. We report here further studies on antibodies in both maternal and cord sera in Nigerians who are continuously exposed to malarial infection.

Materials and Methods

Maternal blood samples and their corresponding cord bloods were collected immediately from apparently healthy Nigerian village women at term; they were not receiving antimalarial therapy. All samples were collected during the peak period of the heaviest rain falls (June to August), which coincides with the peak of the malarial season.

Fluorescent antibody titres. The malarial fluorescent antibody titres of the samples were determined according to the indirect fluorescent antibody technique of Kuvín *et al.* (1962) and Voller (1962). The fluorescent antibody titres of the maternal sera ranged from 1280 to 5120, and the corresponding titres for the cord sera ranged from 640 to 2560.

Immunoelectrophoresis. Each of the maternal sera and their corresponding cord sera were subjected to immunoelectrophoresis in agar gel, using veronal buffer, pH 8.6, ionic strength 0.05, and the technique of Grabar and Williams (1953), as modified by Scheidegger (1955). In each case the maternal serum was placed in the top well and the cord serum was placed in the bottom well. Human antiserum raised locally in rabbits was used to develop the immunoprecipitin lines.

Fractionation of sera by Sephadex G-200 chromatography. 5 ml. of each serum, with 750 mg. sucrose added, was fractionated separately by passage through a column (2.7×150 cm.) of Sephadex G-200. The column was equilibrated and eluted with 0.1M Tris-HCl in 0.5M NaCl pH 8.0 containing 0.1% sodium azide. The flow rate was adjusted to 15–20 ml./hr., and 5 ml. fractions were collected by means of an LKB fraction collector equipped with a uvicord assembly set at 259 $m\mu$ and a recorder (LKB produkter, AB, Stockholm, Sweden). The optical density of each collected fraction was obtained at 280 $m\mu$ to determine the protein content. The effluents were pooled and lyophilised, and reconstituted in 0.5 ml. of ion-free water. The presence of malarial antibody in the fractions was investigated by the indirect fluorescent antibody technique. Fluorescence from each sample was graded as positive (+), weak positive ($\pm$), and negative (–).

Results

Immunoelectrophoresis showed that the maternal sera had the usual precipitin lines. All the cord sera had IgG precipitin lines but only 40% had IgM lines. No IgA was detected in any of the cord sera. Fig. 1 shows a typical immunoelectrophoresis of maternal and cord sera with IgM. Fig. 2 and 3 show the elution patterns obtained when maternal and cord sera were each fractionated on Sephadex G-200 columns. The distribution of malarial antibody, as detected by the fluorescent antibody tests on the lyophilised fractions, occurred in the IgG which is pre-

FIG. 1.—Immunoelectrophoretic pattern of maternal serum (top well) and cord serum (bottom well). Precipitin lines were developed with rabbit anti-whole human serum. Note the presence of a weak IgM precipitin line in the cord serum.

dominant in the second peaks of both the maternal and cord sera chromatograms.

Discussion

The malarial antibody activity was located in the IgG fraction of both the maternal and cord sera. There was no detectable antibody activity in the IgM fraction. The studies of Abele *et al.* (1965)

suggested that 19S macroglobulin antibodies (IgM) as well as 7S γ -globulin antibodies (IgG) are formed during the course of primary malarial infection. They observed an increase in serum IgM precipitin line which coincided closely with the appearance of malarial antibodies in experimentally induced malaria. Tobie *et al.* (1966), using a quantitative immunoplate method, also showed that

FIG. 2.—Sephadex G-200 elution pattern of maternal serum, showing the distribution of malarial antibody in the fractions, as determined by the fluorescent antibody technique. +, positive fluorescence; $\pm$, weak fluorescence; —, negative fluorescence.

FIG. 3.—Sephadex G-200 elution pattern of cord serum, showing the distribution of malarial antibody in the fractions as determined by the fluorescent antibody technique. +, positive fluorescence; ±, weak fluorescence; —, negative fluorescence.

in the early stages of malarial infections the serum usually contained high IgM. The latter investigators noted that during the secondary response the malarial antibody activity appeared to be confined to the IgG fraction. Nigerians living in an endemic malarial area are subjected to repeated malarial infection, and therefore exhibit an immune reaction characteristic of secondary response.

The IgM detected in cord sera by the sensitive diffusion technique is probably of fetal origin, since IgM does not cross the human placenta. Van Furth, Schuit, and Hijmans (1965) have shown IgM-producing cells in the spleen of the 20-week-old human fetus, and McFarlane and Udeozo (1968) have shown that Nigerian cord sera does contain IgM in significant concentration. It is not clear whether the fetally produced immunoglobulin has antibody activity. However, Eichenwald and Shinefield (1963) showed that in the serum of a 20-week-old human fetus suffering from congenital toxoplasmosis, the IgM level was higher than that of the mother, and that the level of the IgM was related to the antibody activity of the cord serum. They also noted that cord blood from fetuses which did not have toxoplasma infection, but were born of mothers with high toxoplasma antibody titres, showed antibody only in the IgG fractions and none in the IgM. A haemagglutinating activity present in the macroglobulin fraction of cord blood in the absence of such activity in maternal sera has also been shown by Epstein (1965).

Since only IgG is known to cross the placenta, it appears that most of the demonstrable malarial antibody in cord blood was passively acquired. The presence of these IgG antibodies may be related to the resistance to malaria infection possessed by the newborn African and probably accounts for the rarity of congenital malaria in the indigenous population of endemic areas (Covell, 1950; Bruce-Chwatt, 1952). The protective role of these passively acquired antibodies in the neonate and the mechanism of their antiparasitic activity merits further investigation.

Summary

The distribution of malarial antibody in the various serum fractions of Nigerian maternal sera and corresponding cord sera was investigated. Most of the malarial antibody, as detected by the fluorescent antibody technique, was located in the IgG fractions of both the maternal and cord sera. Fetal malarial antibody appears to be passively acquired from the mother.

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