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Detection of Anti-Neutrophil Cytoplasmic Antibodies after Acute *Plasmodium falciparum* Malaria

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Four of 30 patients with *Plasmodium falciparum* infection in Bangkok, Thailand, were positive for anti-neutrophil cytoplasmic antibodies by indirect immunofluorescence 1 month after antimalarial therapy. No myeloperoxidase, proteinase 3, lactoferrin, or elastase reactivity was found. Since no evidence of vasculitis was seen in these patients, anti-neutrophil cytoplasmic antibody production in malaria-infected susceptible patients probably represents a secondary response, indicating neutrophil activation.

Antibodies directed against cytoplasmic constituents of the neutrophilic granulocytes (anti-neutrophil cytoplasmic antibodies [ANCA]) have been described as markers for systemic vasculitis (5, 6, 9, 14, 15). Malarial infection may lead to a broad range of immune phenomena, including production of autoantibodies, polyclonal B-cell activation, and immunosuppression (1, 12, 13, 25, 30). Recently, a dramatic rise in the levels of immunoglobulin M (IgM) and IgG autoantibodies possessing characteristics similar to those of natural autoantibodies (2, 21, 28), i.e., polyreactivity and recognition of a large number of self-antigens, has been reported. Since vascular inflammation occurs in both ANCA-related diseases and malaria, we studied the prevalence and specificity of ANCA in patients with acute *Plasmodium falciparum* infection in Bangkok, Thailand. In addition, we examined levels of soluble endothelium-associated molecules (soluble E-selectin or soluble endothelial leukocyte adhesion molecule 1 [sELAM-1], soluble vascular cell adhesion molecule 1 [sVCAM-1], and soluble intercellular adhesion molecule 1 [sICAM-1]) in serum to evaluate a potential relationship between autoantibody production and endothelium inflammation (32).

Patients with malaria were between 16 and 49 years old, had a single infection with *P. falciparum*, and reported no ingestion of chemotherapeutic drugs during the preceding 28 days. Thirty healthy, age- and sex-matched subjects with negative blood smears and no symptoms or signs of infection were the healthy controls. In addition, sera of 15 patients with gram-negative septicemia were studied. The patients were monitored as previously described (30–32). ANCA were screened with ethanol-fixed human granulocytes (Inova Inc., San Diego, Calif.). Screening took place at a concentration of 1:40 with polyspecific goat anti-human Ig conjugated to fluorescein isothiocyanate (Sigma, Munich, Germany). Positive sera were additionally evaluated on formalin-fixed granulocytes (Inova). To determine Ig isotype specificity, positive sera were analyzed with IgG- and IgM-specific fluorescein isothiocyanate-conjugated goat anti-human Ig. All sera were tested for the presence of proteinase 3 (PR-3) and myeloperoxidase (MPO) autoantibodies by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Elias, Freiburg, Germany). To elucidate reactivities against lactoferrin and elastase, positive sera were "blocked" by adding both proteins in excess prior to dilution. Potential antibody-antigen complexes were precipitated with polyethylene glycol (24). Thereafter, sera were evaluated on ethanol-fixed granulocytes. Concentrations of sICAM-1, sELAM-1, and sVCAM-1 in serum were determined as previously described (31, 32). The mean serum sELAM-1 level in normal controls was 21.5 ± 10.1 ng/ml, the mean serum sVCAM-1 concentration in healthy subjects was 12 ± 4 ng/ml, and the mean serum sICAM-1 level was 160 ± 47 ng/ml. To compare the results from patients and controls, Student's *t* test was used. For correlations, Pearson's correlation matrix was used. Values are expressed as means ± standard deviations. All of the analyses were two-sided, and differences with a P value of less than 0.05 were considered significant.

All patients had acute falciparum malaria with an average temperature on admission of 38.4°C. No symptoms or signs suggestive of vasculitis were observed. The mean parasite clearance time was 63 ± 18 h. Fever persisted on average for 45 ± 44 h after the start of treatment. No recrudescence occurred. The geometric mean parasite count prior to treatment was 69,450 asexual parasites per μl. Fever clearance correlated with parasite clearance ($r = 0.784$, $P < 0.05$). Twenty-eight days after therapy, 4 of 30 patients were IgG-ANCA positive. None of these patients exhibited antibodies against MPO, PR-3, lactoferrin, or elastase. All IgG-ANCA-positive patients (on day 28) had IgM-ANCA prior to antimalarial treatment (day 0). None of the local healthy controls, none of the patients with septicemia, and none of the patients with malaria on day 0 were IgG-ANCA positive. The levels of endothelium-associated adhesion molecules in serum were significantly elevated in patients prior to treatment (sICAM-1, 703 ± 241; sVCAM-1, 88 ± 14; and sELAM-1, 78 ± 18 ng/ml) compared with those of normal controls ($P < 0.001$ for all). Serum sELAM-1 levels dropped to normal levels on day 7, whereas serum sICAM-1 and sVCAM-1 levels dropped steadily during reconvalescence but remained elevated even 28 days after therapy (Table 1).

This article describes IgG antibodies to the neutrophilic cytoplasm in patients with complicated *P. falciparum* malaria 28 days after treatment. Prior to therapy, IgM reactivity was seen in all four patients developing IgG-ANCA. These IgM

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TABLE 1. Serological findings from ANCA-positive patients on days 0 and 28[a]

Patient	No. of parasites/μl		Test result or titer[b] of anti-human antibody type						Concn in serum[c] (ng/ml) of:					
			Polyspecific		IgG specific		IgM specific		sELAM-1		sICAM-1		sVCAM-1	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
A	601,750	0	Neg	1:80	Neg	1:80	1:80	1:40	167	27	1,346	389	202	44
B	480,200	0	Neg	1:40	Neg	1:40	1:40	Neg	184	33	1,475	404	189	39
C	455,800	0	Neg	1:40	Neg	1:40	1:80	1:40	199	29	1,298	376	224	33
D	576,850	0	Neg	1:40	Neg	1:40	1:40	Neg	206	31	1,379	298	267	47

[a] All patients tested negative for anti-MPO and -PR-3 antibodies on both days.
[b] Titers were determined by cytoplasmic staining of formalin-fixed slides. Neg., negative.
[c] Determined by ELISA.

antibodies might represent malaria infection-driven polyreactive Ig production (IgM), as has been described previously (1). However, no follow-up data were provided for this study (7, 26). We also tested for ANCA in the sera of patients with septicemia (prior to therapy and 28 days after therapy [30]), but no ANCA were seen. IgM-ANCA production during acute malaria might predispose a patient to develop IgG-ANCA reactivity, and it might in part explain the controversy of whether ANCA are found in infections (5, 17). The immunofluorescence pattern demonstrated a fine-granular cytoplasmic staining (Fig. 1 and 2). Furthermore, the MPO and PR-3 ELISAs yielded negative results, suggesting that no "classical" ANCA are produced after the acute malaria attack. By contrast, in patients with invasive amoebiasis, 75% of ANCA-positive sera demonstrated PR-3 specificity (23). The ANCA-positive patients had a very high parasite density (>400,000/μl), and very high levels of endothelium-associated adhesion molecules were found compared with those of ANCA-negative patients and previously reported cases (31, 32; Table 1). The endothelium-associated forms of these molecules are receptors for parasitized erythrocytes (PE), facilitating the sequestration of PE in deep vascular beds. The sequestration of PE plays a crucial role in the pathogenesis of malaria, since on one hand, the adhesion of PE to microvascular endothelium may be a requirement for the survival of *P. falciparum* in vivo, while on the other hand, it may trigger a cascade of deleterious events, including the induction of toxic inflammatory mediators, metabolic disturbances, and tissue anoxia, leading to complicated and cerebral malaria (3, 11, 16, 18–20, 22, 29, 33). The presence of granulocyte cytoplasmic antigens on endothelial cells has been demonstrated, and ANCA increased neutrophil adhesion to the vascular endothelium (4). Further, cytokines such as the tumor necrosis factor are known to translocate ANCA-reactive proteins from primary granules to the neutrophil cell surface, thereby exposing the antigen to the patient's immune system (4). Moreover, a genetic susceptibility to ANCA-associated diseases has been demonstrated (8, 27), suggesting that only susceptible individuals may develop ANCA after an infectious or inflammatory stimulus. We conclude that *P. falciparum* malaria is capable of inducing ANCA in susceptible patients. Since the IgG-ANCA-positive patients showed no clinical evidence of vasculitis, the presence of ANCA probably

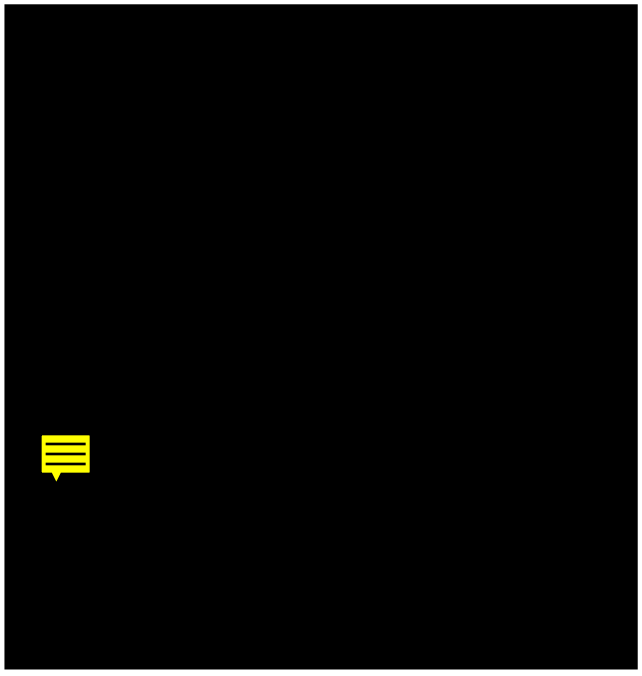

FIG. 1. Immunofluorescence staining of serum sample of patient A on day 0. Magnification, ×400.

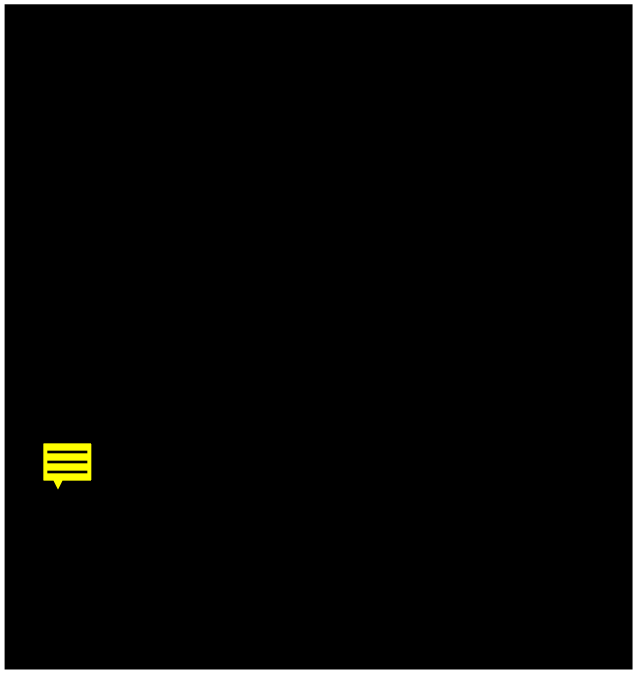

FIG. 2. Immunofluorescence staining of serum sample of patient A on day 28. Magnification, ×400.

represents a secondary response, indicating neutrophil activation, as may also occur in classical ANCA-associated diseases (10, 17).

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