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ANTIGENIC CROSS-REACTIVITY OF *PLASMODIUM FALCIPARUM* ANTIGENS EXPRESSED IN *ESCHERICHIA COLI*

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Phage clones were isolated from a P. falciparum *genomic DNA library in the vector lambda gt11 that were reactive with sera from malaria patients. Characterization of the clones by plaque antibody selection and DNA hybridization lead to a definition of a family of cross-reacting parasite antigens.*

The worldwide recrudescence of malaria reflecting the failure of current chemotherapy and the appearance of mosquito vectors resistant to insecticides has lead to the search of other methods of controling the disease such as immunoprophylaxis. Control of parasitemia by passive transfer of sera from adults living in endemic areas (Cohen, McGregor & Carrington, 1961) demonstrated the importance of antibodies in the mechanisms of immunological protection. Since the pathology of malaria is mostly due to its blood stages, many laboratories concentrated efforts on characterizing antigens of these stages, possibly involved in the induction of protective antibodies. The approaches have been rather empirical such as the use of crude or fractionated extracts of parasite material in immunization of animal models and analysis of the proteins recognized by the sera of immunized animals (for a review see Dubois et al., 1986). Another approach has been the screening of libraries in *E. coli* of parasite genetic material with sera from malaria patients presumably containing protective antibodies (Kemp et al., 1983). This approach has been met with some success as a relative degree of protection against *P. falciparum* infection was achieved by immunizing monkeys with the recombinant polypeptide RESA (Coolins et al., 1986) the clone of which was isolated in such a screening. We also adopted the latter approach and screened a library of *P. falciparum* genomic DNA with sera from malaria patients from endemic areas. Preliminar characterization of the positive clones by plaque antibody select yielded two type of clones (Ozaki et al., 1986). One in which the purified antibodies recognized a single or few polypeptydes in a Western blot of *P. falciparum* protein extract and another in which the purified antibodies unexpectedely recognized a series of multiple components leading to similar patterns of recognition. The last type of clones define a family of antigenically cross-reacting proteins.

MATERIALS AND METHODS

Construction of the *P. falciparum* genomic DNA library on the phage vector lambda gt11 (Young & Davis, 1983), antibody screening and plaque antibody select is described in Ozaki et al. (1986). Phage clones were gridded on a 90mm LB nutrient agar (Miller, 1972) with a fresh lawn of appropriate bacteria and incubated at 37°C overnight. The phages were then blotted onto nitrocellulose filter and prepared for DNA hybridization as described (Ozaki & Cseko, 1984). DNA inserts of clones were purified from the vector on agarose gel and eletroelution and, ^{32}P-labelled by nick-translation (Maniatis, Fritsch & Sambrook, 1982).

RESULTS AND DISCUSSION

The phage clones recognized by antibodies from malaria patients were plaque purified and each plated at about 10^4 phages per 90mm nutrient agar plate. Antibodies were then purified from the sera of malaria patients used in the original screening on the expression induced bacterial cells lysed by the recombinant phage and used to probe a Western blot of parasite protein extract as described (Ozaki et al., 1986). Figure 1 shows the result with various clones in which the purified antibodies give a similar and multiband pattern on a Western blot. The most prominent band is at about 160 kilodaltons (KDa). The antibodies also strongly recognize polypeptides of very high molecular weight (more than 250 KDa). In spite of the similarity of the recognition pattern of the purified antibodies, the clones were found to be different when hybridized to each other

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at the nucleic acid level. At least five clones could be differentiated (Table I) by using various DNA probes. Two probes were used to identify the "clone 11-1". One of 620 base pairs (bp) from the clone pPF11-1 consisting of only repetitive sequences (Koenen et al., 1984) and another of about 10 kilobases (kb) from a genomic clone (Scherf et al., unpublished) isolated from a phage EMBL4 (Frischauf et al., 1983) *P. falciparum* genomic DNA library with the 620bp probe and subcloned into pKY2700 (Ozaki et al., 1980). No additional clones hybridized to the 10kb other than those which hybridized to the 620bp probe. Other clones were classified by hybridizing the DNA insert of each clone to the others. H54, H102 and H139 were in this way differentiated and still few clones remain to be classified.

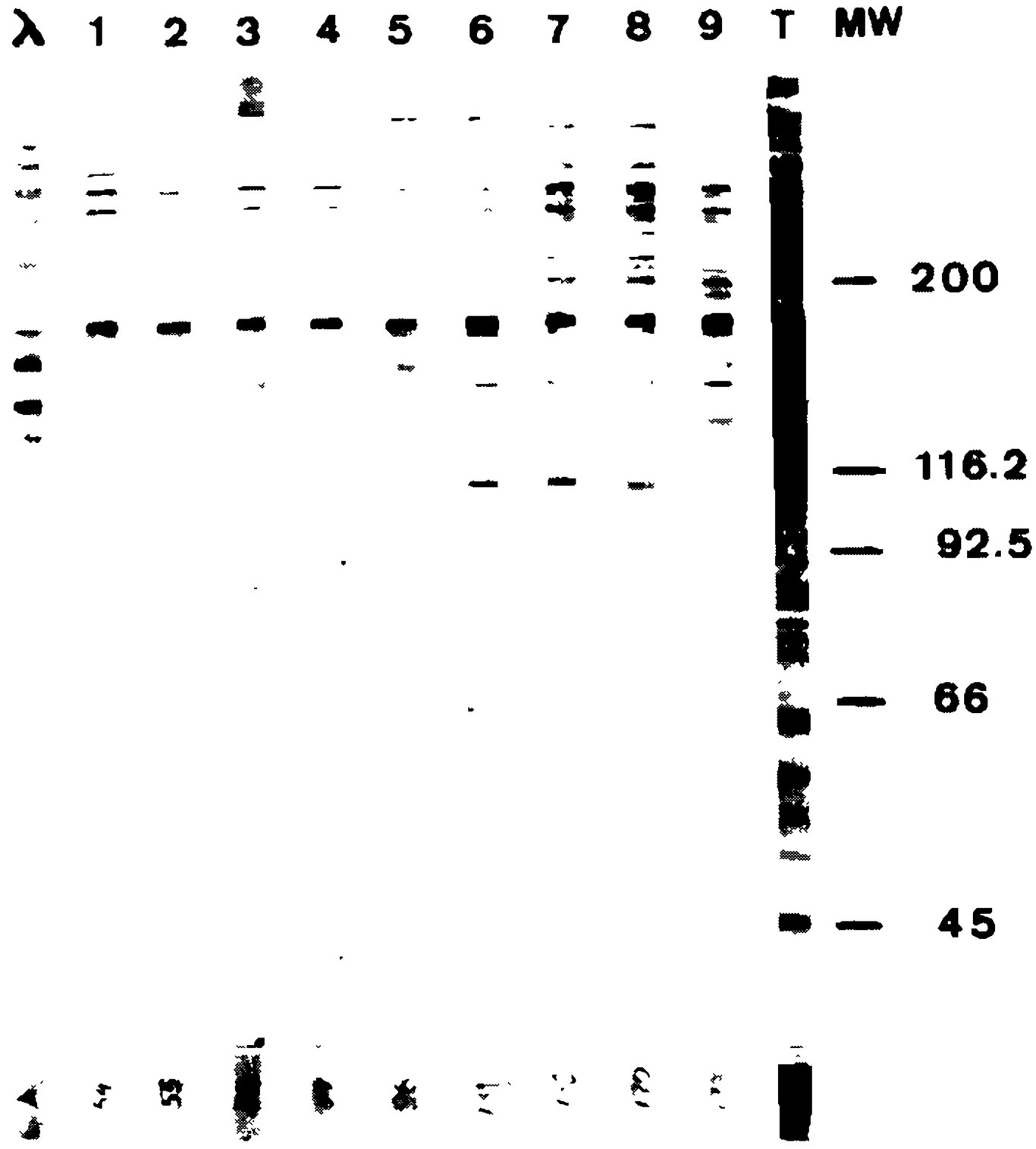

Fig. 1: Analysis by Western blot of *P. falciparum* protein extract with antibodies purified from human sera on plaques of recombinant phages expressing parasite antigens. Strips of nitrocellulose from the Western blot were incubated with antibodies purified on phage plaques of lambda gt11 ( ); on phage plaques of individual clones (1 to 9); and with the original human sera (T). MW, molecular weight markers.

TABLE I

Classification of *P. falciparum* phage clones postive to human sera from malaria patients according to their hybridization to different DNA probes.

Clone	Reference of probe
"11-1"	Koenen et al. (1984)
H54	This work
H102	This work
H139	This work

Other examples of immunological cross-reactivity between different antigens in *P. falciparum* have been reported (Wahlgren et al., 1986; Mattei et al., 1986). The clones described here express fusion proteins that have cross-reacting epitopes. However, when animals are immunized with purified recombinant proteins, the immune sera obtained no longer detect cross-reactivity

between different parasite components (data not shown). Cross-reactive antibodies may thus only result from the complex interaction of multiple cross-reacting epitopes with the host immune system during malarial infections. Another possibility is that the polyclonal activation which occurs in malaria (Rosenberg, 1978) may play a role in the appearance of this vast repertoire of cross-reacting antibody specificities.

Flooding the immune system with multiple cross-reacting epitopes (many of which may be repeated) could result in the absorption of a large population of antibodies. In the light of the network theory (Jerne, 1974), this should have a major consequence on the regulation of the host's immune system.

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