that the antibodies to Thy-1.1 exerted some of their protective effects by modifying the T cell system (for example, by elimination of suppressor cells) of the host.

The effectiveness of antibodies against differentiation antigens for the treatment of lymphoid tumors is of considerable clinical importance, because human leukemias also are known to contain differentiation antigens on the cell surface that are analogous to those described in mouse tumors (*8*). The results described here should add impetus to the development of cell lines that produce monoclonal human antibodies against similar types of antigens on human tumor cells.

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Hybridoma Produces Protective Antibodies Directed Against the Sporozoite Stage of Malaria Parasite

Abstract. *Hybrid cells secreting antibodies against sporozoites of* Plasmodium berghei *were obtained by fusion of plasmacytoma cells with immune murine spleen cells. The monoclonal antibodies bound to a protein with an apparent molecular weight of 44,000 (Pb44), which envelopes the surface membrane of sporozoites. Incubation of sporozoites in vitro with antibodies to Pb44 abolished their infectivity.*

Studies on immunity induced by the sporozoite stage of mammalian malaria, carried out in rodents, rhesus monkeys, and man, have shown that total protection can be achieved by repeated bites of infected mosquitoes or by intravenous injections of x-irradiated sporozoites (*1*). Antibodies to sporozoites also occur in the serum of more than 90 percent of the adults living in an area of hyperendemic malaria where they are frequently exposed to the bites of *Plasmodium falciparum*-infected mosquitoes (*2*).

The antigens involved in these immune responses, which are strictly stage-specific, have not been characterized. Rodents immunized with sporozoites produce antibodies that react in vitro with the membrane of the parasite, as demonstrated by circumsporozoite precipitation (CSP) (*3*), indirect immunofluorescence (*4*), and sporozoite neutralizing activity (*1*). Stage-specific antigens on the surface of rodent, simian, and human malaria sporozoites have also been found by ultrastructural observations (*5*) and serological tests (*4*). In experiments aimed at the characterization of the surface antigens of sporozoites of *P. berghei*, a parasite of rodent malaria, a membrane component with molecular weight close to 44,000 (Pb44) was specifically immunoprecipitated by the serum of vaccinated mice (*6*).

Here we report that we have hybridized sporozoite antibody-producing murine spleen cells with plasmacytoma cells; the hybrid cells produce antibodies to Pb44 that mediate the CSP and immunofluorescence reactions and have sporozoite neutralizing activity.

Mice of strain BALB/c, immunized by exposure to repeated bites of γ-irradiated *P. berghei*-infected mosquitoes, served as the source of spleen cells. Hybridization was carried out according to the method of Köhler and Milstein (*7*), with 1.4×10^7 plasmacytoma P3U1 cells (κ chain secretor) and 1.4×10^8 spleen cells collected from a mouse 4 days after the last mosquito bite. The production of sporozoite antibodies by individual hybridoma cultures was assessed by indirect immunofluorescence (*4*), glutaraldehyde-fixed sporozoites being used as antigens, and also by the CSP reaction (*3*) with viable parasites.

The supernatants of 600 cultures were screened, and five were found to produce sporozoite antibodies 2 weeks after the fusion procedure. However, only one hybrid cell line (3D11) was successfully expanded; this line has been maintained in Dulbecco's modified Eagle medium containing 10 percent fetal calf serum (*8*). The hybrid cells were also inoculated intraperitoneally into BALB/c mice, which resulted in the rapid formation of ascites containing high concentrations of sporozoite antibodies (Table 1). The CSP and immunofluorescence titers obtained with the serum and the ascitic fluid of hybridoma-inoculated mice were from 10^2 to 10^3 times higher than the titer of the serum of mice immunized repeatedly with irradiated sporozoites. The monoclonal antibodies were capable of neutralizing sporozoites in vitro, abolishing their infectivity. In four experiments, *P. berghei* sporozoites were incubated for 45 minutes at room temperature either with the 3D11 culture fluid or with

Table 1. Titer of *P. berghei* antisporozoite antibodies produced by hybridoma cells and by sporozoite-immunized mice. Viable and glutaraldehyde-fixed sporozoites were used as antigen for the CSP and immunofluorescence reactions, respectively.

Source of antibody	CSP titer	Immunofluorescence titer
Hybridoma		
Culture fluid*	1 : 16	1 : 2,048
Serum from mice with solid tumors†	1 : 400	1 : 1,160,000
Serum from mice with ascites†	1 : 1,600	1 : 800,000
Ascites‡	1 : 1,600	1 : 1,600,000
Mice immunized with *P. berghei* sporozoites	1 : 32	1 : 4,000

*Culture fluids were titrated at the time of maximum cell growth. †Serum was collected from BALB/c mice 9 to 10 days after they were inoculated subcutaneously or intraperitoneally with 7×10^6 3D11 cells. ‡Ascitic fluid was obtained from BALB/c mice 1 to 2 weeks after they were inoculated with 7×10^6 3D11 cells.

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the serum of mice inoculated with hybrid cells, and then injected into a total of 30 mice, each challenged with 10^4 parasites. Only one of these mice developed parasitemia. In contrast, of the control group that had been given sporozoites incubated in normal mouse serum, 14 out of 15 animals developed patent lethal infections.

The interaction of 3D11 antibodies with *P. berghei* sporozoites was also observed at the ultrastructural level. Sporozoites were incubated with 3D11 culture fluid at 37°C for 30 minutes, washed, and fixed in 2.5 percent glutaraldehyde, 0.1*M* cacodylate buffer, *p*H 7.3, and 4 percent sucrose. This preparation was postfixed in 1 percent osmium tetroxide for 1 hour, dehydrated, and then embedded in Epon 812. The sections, examined with a Siemens Elmiskop 101 electron microscope, revealed an amorphous deposit enveloping the external membrane of the parasites (Fig. 1). The immunofluorescence patterns also showed a uniform bright linear staining surrounding the sporozoites. No deposit was observed in controls incubated with culture fluid from P3U1 cells.

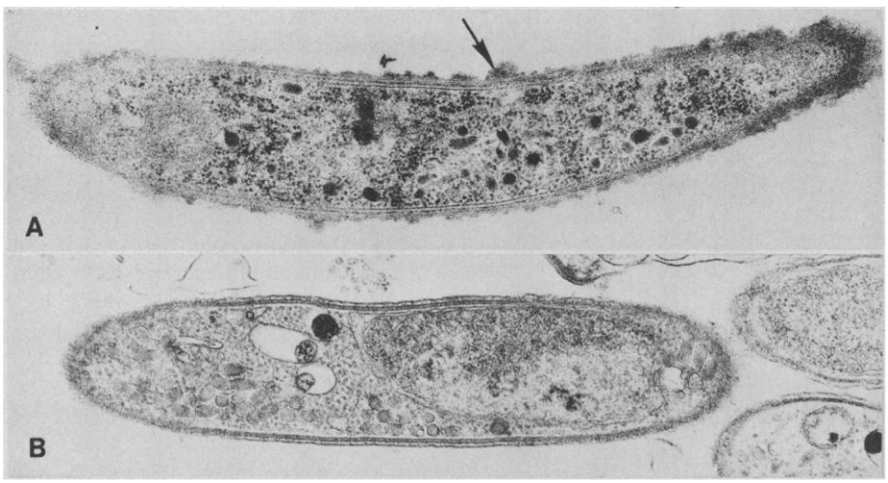

Fig. 1. Electron micrograph of sporozoites of *P. berghei*. (A) Sporozoites were incubated with 3D11 hybridoma culture fluid at 37°C for 30 minutes, and treated as described in the text. Note the electron-dense surface coat (arrow) (×20,000). (B) Sporozoites before treatment with 3D11 antibodies, showing no surface coat (×26,000).

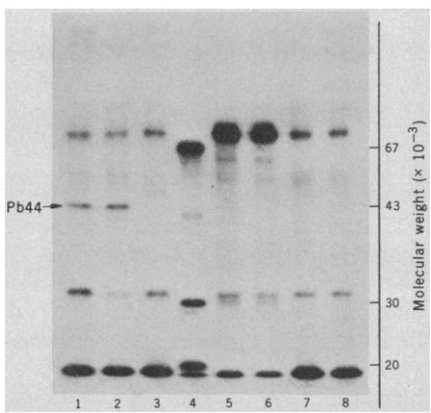

Fig. 2. Radioautograms of *P. berghei* sporozoite preparations subjected to electrophoresis on SDS-polyacrylamide gel. Sporozoites were purified from the salivary glands by successive passage through columns containing Sepharose-6MB that had been covalently coupled (i) to thyroglobulin and (ii) to hog gastric mucin, and then incubated with excess concanavalin A and wheat germ agglutinin. Most of the bacteria and mosquito debris adhered tightly to both columns. Recovery of sporozoites was between 70 and 90 percent. The partially purified sporozoites were labeled with ^{125}I by the lactoperoxidase method, dialyzed overnight to remove free iodine, and then disrupted with the French pressure cell. The following inhibitors of proteolysis were added: diisopropyl fluorophosphate (4 m*M*), aprotinin (two trypsin-inhibiting units per milliliter), and the protease inhibitors of bacterial origin antipain and leupeptin (25 μg/ml). The supernatant obtained by centrifugation at 10,000*g* and containing more than 80 percent of the counts was used as the parasite extract throughout the experiments. The same procedure was used for purifying and labeling material from normal mosquito salivary glands. Sporozoite extracts were immunoprecipitated with the various antibodies, then the immune complexes were bound to a 10 percent *Staphylococcus aureus* (Staph-A) suspension and eluted with a mixture containing 2 percent SDS, 10 percent glycerol, 10 percent 2-β-mercaptoethanol, and 6*M* urea. The eluate was subjected to electrophoresis on 10 percent SDS-polyacrylamide gel and the gels were radioautographed after fixation and drying. Molecular weight markers, shown in track 4, were bovine albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (20,000). Sporozoite extracts were immunoprecipitated with antiserum to *P. berghei* from mice immunized by the bite of x-irradiated infected mosquitoes (track 1), 3D11 culture fluid (track 2), and antiserum from mice bitten by noninfected mosquitoes (track 3). Normal mosquito extracts were treated with antiserum to *P. berghei* (track 8) or with 3D11 supernatant (track 7). The total extracts, from sporozoite-infected and normal mosquitoes (tracks 5 and 6, respectively), give very similar patterns, suggesting that most bands may not be sporozoite-derived. A single band, with an apparent molecular weight of 44,000, was specifically immunoprecipitated by 3D11 or by the antiserum to *P. berghei* from preparations containing sporozoites, but not from normal mosquito extracts (*15*).

To identify the molecule that interacted with the 3D11 antibodies, we partially purified sporozoites of *P. berghei* derived from the salivary glands of infected *Anopheles* mosquitoes and then labeled them with ^{125}I by the method of lactoperoxidase catalyzed iodination (*9*). After overnight dialysis against phosphate-buffered saline, the parasite preparation was disrupted with a French pressure cell and centrifuged in a Beckman Microfuge B for 3 minutes at about 10,000*g*. More than 80 percent of the counts were recovered in the supernatant, which was designated as parasite extract and used in all subsequent steps.

Immunoprecipitation was carried out by incubating portions of the parasite extract at room temperature for 10 minutes with the 3D11 culture fluid and with either antiserum to *P. berghei* sporozoites or normal mosquitoes obtained from mice subjected to repeated bites of infected and noninfected mosquitoes, respectively. The immunocomplexes were bound to a 10 percent protein A–*Staphylococcus aureus* suspension (*10*). After several washings, the antigens were eluted from the staphylococcal preparation and then electrophoresed in a 10 percent sodium dodecyl sulfate (SDS)-polyacrylamide gel (*11*), under reducing conditions. The gel was dried and exposed to an X-omat R film XR-2 at −70°C, for radioautography. An extract from uninfected mosquitoes, which served as control, was prepared by dissecting uninfected mosquito salivary glands and processed in the same way.

The total extracts, originating either from infected or noninfected salivary glands, gave almost identical gel patterns (Fig. 2, tracks 5 and 6). Only one band, of molecular weight 44,000 (Pb44), was specifically immunoprecipitated by the 3D11 hybridoma culture supernatant or by the antiserum to *P. berghei* (tracks 2 and 1, respectively). As controls we used the *P. berghei* extract immunoprecipitated with an antiserum to normal mosquito (track 3) or with normal mouse serum (not shown). The Pb44 band was not immunoprecipitated from extracts of normal mosquito salivary glands with

the 3D11 hybridoma or with the antiserum to *P. berghei* (tracks 7 and 8).

The Pb44 antigen was not detected on other developmental stages of *P. berghei*, by indirect immunofluorescence when monoclonal antibodies were used. Also, antibodies to sporozoites of two simian malaria parasites, *Plasmodium knowlesi* and *P. cynomolgi*, did not immunoprecipitate Pb44. This antigen is therefore probably stage- and species-specific.

Whether Pb44 constitutes the major, or even the single antigen on the outer membrane of the sporozoites, as is the case of the antigen from the surface coat of various African trypanosomes (*12–14*), is unknown. The availability of large amounts of this monospecific sporozoite antibody produced by hybrid cells will greatly facilitate its purification and subsequent characterization, and contribute to clarification of its role in protective immunity in vivo. Experiments show that mice bearing hybridoma solid tumors are resistant to sporozoite challenge and that their serum mediates passive protection (*8*).

Antibodies to sporozoites have been detected in humans (*2*) in spite of the small number of parasites inoculated by a mosquito bite and the brief passage of the parasites through the host's blood stream. This suggests that the sporozoites have a high degree of immunogenicity and could be used in the development of a malaria vaccine.

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Sperm-Egg Interaction: Evidence for Boar Sperm Plasma Membrane Receptors for Porcine Zona Pellucida

Abstract. *Freshly ejaculated, noncapacitated boar sperm bind rapidly and in large numbers to pig egg zona pellucida in vitro. In the present study, the number of sperm bound decreased sharply when sperm motility was lowered by energy poisons or by reducing the temperature. Highly motile sperm from humans, guinea pigs, and rats, added at concentrations ten times higher than control sperm, did not bind to the porcine zona. At the same high concentration, a small number of hamster and bull sperm bound to the zona. Binding of boar sperm to the zona pellucida was blocked almost completely by diluted whole antiserum to sperm plasma membranes and by univalent (Fab) antibody to these membranes. When antibody to sperm plasma membrane was first absorbed with plasma membrane vesicles, sperm binding was not inhibited. These results provide direct evidence for the existence of sperm plasma membrane receptors for the zona pellucida of the pig.*

Gamete interactions have been studied in detail in some species of invertebrates, and a species-specific sperm protein, thought to be involved in the adhesion of sea urchin sperm to the egg surface, has been isolated (*1, 2*). In mammals, species specificity has been reported to reside in the ability of sperm to bind with the zona pellucida (*3*), an ability that is lost in the presence of antibody to eggs (*4–7*) or to whole sperm (*8*). An important inference that can be made from these studies is that receptors for the zona exist on the surface of the spermatozoon; the existence of such receptors, however, has not been directly demonstrated. In this report we present the results of experiments which characterize the binding of ejaculated boar sperm to the zona pellucida of porcine oocytes and show that this binding is inhibited by antiserum and univalent antibodies to boar sperm plasma membranes.

Ovaries obtained from sows and gilts after slaughter were minced with a sharp scalpel to free oocytes (*9*), which were collected in nylon screens (*10, 11*). Cumulus-free eggs in various stages of maturation (*11, 12*) were obtained by briefly rinsing the eggs with sodium citrate during isolation. Freshly ejaculated boar sperm were washed twice in a medium containing tris-HCl (20 m*M*), NaCl (120 m*M*), KCl (5 m*M*), $MgCl_2$ (1 m*M*), $CaCl_2$ (3 m*M*), glucose (5 m*M*), sodium pyruvate (3 m*M*), and bovine serum albumin (2 percent); the *p*H was 7.4 at 21°C.

In each experiment, about 50 eggs were added in a small volume (about 20 μl) to 0.2 ml of a sperm suspension (0.2×10^4 to 0.6×10^4 cells) in buffer at room temperature and incubated in a petri dish for 60 minutes. Pasteur pipettes, drawn out to a narrow bore slightly larger in diameter than the eggs (about 150 μm), were used to transfer eggs to fresh, sperm-free medium. This was repeated three times to remove unbound or loosely bound sperm. [This method proved more reproducible and practical than a centrifugation method recently introduced to measure the extent of mouse sperm-egg binding (*13*).] Eggs were fixed in formalin, mounted on glass slides, and the number of sperm bound per egg counted by using phase-contrast microscopy. At least 20 eggs were counted on each slide.

Membrane vesicles from boar sperm were obtained by nitrogen cavitation and sucrose density centrifugation as described by Gillis *et al.* (*14*). Electron micrographs of these preparations and enzyme marker assays indicated that the plasma membranes were highly purified and free from other possible sperm membrane contaminants (*14*). Antiserums to these membranes were prepared by subcutaneous injection of 50 to 100 μg of

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