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8. A Hach model OX-2P field kit (a modified Winkler method) and a YSI model 57 dissolved oxygen meter were used to measure the DO contents in our ground waters. The YSI meter is accurate to 0.1 mg/liter and the Hach field kit to 0.2 mg/liter. Measurements of DO (12 replicates) at a deep well in the Vekol Valley, Ariz., made with the Hach kit gave a mean of 4.3 mg/liter with a standard deviation of 0.2 mg/liter. This compared with a value of 4.1 ± 0.1 mg/liter, measured with the YSI meter on water from the same well. C. E. Boyd [*J. Environ. Qual.* **6**, 381 (1977)] compared the Hach field kit DO values with standard laboratory methods; in the range of interest to us (3 to 7 mg/liter), the Hach kit results were only 3 to 13 percent higher than those obtained by standard laboratory methods.
9. D. C. Thorstenson, D. W. Fisher, M. G. Croft, *Water Resour. Res.* **15**, 1479 (1979); I. J. Winograd and G. M. Farlekas, in *Isotope Techniques in Groundwater Hydrology 1974* (International Atomic Energy Agency, Vienna, 1974), vol. 2, pp. 69–93. Indirect geochemical evidence that neither our pumping nor our sampling procedures caused the oxygenation of naturally reduced ground waters consists in the virtual absence of iron in all our waters. The dissolved iron content of the southern Nevada ground waters, for example, varies from 15 to 40 μg/liter. If we had aerated moderately reduced [*Eh* (oxidation-reduction potential) −100 to +200 mV] waters, we would have expected iron contents—either dissolved or as precipitates on our filters—in the milligrams per liter range, in at least some of our waters, all of which have *p*H values in the range of 6.5 to 8.
10. See Winograd and Pearson (*7*); I. J. Winograd and W. Thordarson, *U.S. Geol. Surv. Prof. Pap. 712-C* (1975); W. W. Dudley, Jr., and J. D. Larson, *U.S. Geol. Surv., Prof. Pap. 927* (1976); I. J. Winograd and G. C. Doty, *U.S. Geol. Surv. Open-file Rep. 80-569* (1980); I. J. Winograd and I. Friedman, *Geol. Soc. Am. Bull.* **83**, 3691 (1972).
11. See Winograd and Pearson (*7*); H. C. Claassen, personal communication.
12. Several nonchemical factors are also likely to influence the DO content of water in this hydrogeologically complex aquifer. These include (i) probable reaeration of recharge water in the shallow subsurface during flow through caverns beneath the principal recharge area, the Spring Mountains; (ii) water temperature and pressure, both functions of the depth of burial of the aquifer; (iii) possible outgassing, or conversely absorption of O_2, in areas where the regional carbonate aquifer is unconfined, as beneath the ridges of the region (*10*); and (iv) mixing, in the central part of the Ash Meadows basin, of Spring Mountains water with deep oxygenated water from another source (*10*).
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20 January 1982; revised 6 April 1982

Induction of Crisis Forms in Cultured *Plasmodium falciparum* with Human Immune Serum from Sudan

Abstract. *Serums from 90 individuals from three areas in Sudan were tested for inhibitory activity against cultures of* Plasmodium falciparum. *In addition to inhibitory activity against merozoite invasion, all of the serums demonstrated, in varying degrees, the ability to retard intraerythrocytic development, leading to crisis forms and parasite deterioration. These retardation factors could be removed by absorption of immune serum with parasite-infected erythrocytes and were demonstrable in purified immunoglobulin fractions. Serum from donors in hypoendemic Khartoum did not retard parasite development.*

Nearly four decades ago, Taliaferro and Taliaferro (*1*) reported that infections of *Plasmodium brasilianum* in *Cebus capucinus* monkeys progressed at a predictable rate and pattern until the host's immune response began to resolve the infection. The parasite's highly synchronous development then became severely retarded, and "crisis forms" of the parasite appeared (*1*). The crisis was characterized by significant changes in the synchrony of the parasite's developmental cycle, a reduced average number of merozoites per segmenter, and a retardation of the periodicity, resulting in many deteriorating schizonts within the infected erythrocytes. Since this early report, the term crisis form has become synonymous with obviously degenerating intraerythrocytic parasites seen in hemoprotozoan infections with *Babesia* and *Plasmodium* sp. (*2*). Experimental induction of crisis forms is not always consistent and, in rodent infections with *Babesia* and *Plasmodium* sp., nonspecific factors associated with *Corynebacterium parvum*, *Mycobacterium bovis* BCG, or endotoxin-stimulated macrophages appear to be important (*3*).

Studies of immunity to primate malaria—including malaria due to *P. falciparum* in man—have demonstrated that serums collected from experimentally infected animals, or from humans living in regions of malaria hyperendemicity, contain humoral factors that inhibit parasite development in vitro (*4*). Since the merozoite is the only extracellular stage of the blood infection, it is especially susceptible to immunologic attack, and numerous studies have confirmed that malaria-immune serum appears to act by blocking invasion of erythrocytes by the merozoites (*5*). Attempts to demonstrate inhibition of intracellular parasite development or to identify additional protective actions for malaria-immune serum have been, up to now, unsuccessful (*6*). One result of these studies has been to emphasize the merozoite as the source of protective antigen. We now report that serums collected from individuals living in malarious regions of the Sudan not only contain merozoite-blocking antibodies, but also cause intracellular parasite deterioration and classical crisis forms in cultures of *P. falciparum*.

We have collected more than 300 serum samples from three different regions in Sudan, and of these, 90 have been tested for parasite inhibition in continuous cultures of *P. falciparum*. Since in some areas, particularly Blue Nile Province, the villagers have access to chloroquine, all serums were dialyzed 1:1000 against RPMI 1640 medium. This procedure removes 98 percent of the chloroquine from serum (*7*). Because dialysis also removes hypoxanthine, a required nutrient not found in RPMI 1640, complete medium was supplemented with hypoxanthine to give a final concentration of $5 \times 10^{-5} M$ (*8*). All serums were heat-inactivated at 56°C for 30 minutes. Parasites of *P. falciparum*, strain FCR_3/Gambian, were synchronized with a modification of the sorbitol method (*9*); cultures were washed with 5 percent (weight to volume) aqueous sorbitol, cultured for 12 hours, washed again with sorbitol, returned to culture for 24 hours, then concentrated to 80 to 90 percent parasitemia by the gelatin–RPMI 1640 method (*10*). This procedure results in highly synchronous schizonts with a 6-hour age differential. The synchronized schizont-infected red cells were diluted to a 0.5 to 1.0 percent parasitemia with freshly washed O+ erythrocytes and dispersed into 96-well microculture plates so that each well received 3 μl of packed erythrocytes. The dialyzed serum was

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diluted with hypoxanthine-supplemented RPMI 1640 medium containing 5 percent normal pooled human serum (used to provide minimum essential nutritional ingredients) to give immune serum titers of 1:2, 1:4, 1:8, and 1:16, depending on the experiment. The microculture plates were incubated at 37°C with the candle-jar technique (*11*). The effect of the immune serum was determined by microscopic examination of Giemsa-stained blood films usually made at 48 hours. Since the synchronous schizont-infected red cells released their merozoites within 12 hours, in some experiments the medium in the microplates was changed at 18 hours so that wells containing normal serum during the invasion phase would contain immune serum for the rest of the parasite developmental cycle. Conversely, some parasites exposed to immune serum during invasion of the erythrocytes were switched to normal serum for the developmental phase. In other experiments, the immune serum was replaced with purified immunoglobulins obtained by three consecutive salt fractionations with 33.3 percent ammonium sulfate followed by chromatography on DEAE–Sephadex A-50 according to standard procedures. Once it became apparent that immune serums were retarding intracellular parasite development, some were absorbed three times with one-tenth volume of trophozoite- and schizont-infected red cells at 37° and 4°C for 30 and 120 minutes each, respectively, and tested in parallel with unabsorbed samples of the same serum. Normal, nonimmune serum was also absorbed against parasite-infected erythrocytes, and immune serums were absorbed against uninfected erythrocytes to remove any heterophilic antibodies.

In cultures containing normal nonimmune serum, the synchronized schizonts usually released their merozoites 6 to 12 hours into the experiment, the result being a six- to eightfold increase in parasitemia. At 48 hours into the experiment, which represents 30 to 42 hours of the subsequent parasite cycle, the cultures averaged 65 to 80 percent multinucleated schizonts or segmenters, 20 to 35 percent mature trophozoites, and less than 2 percent ring forms (Fig. 1, A and B). The concentration of dialyzed normal serum had little effect on the rate of parasite development or the final parasitemia. In cultures supplemented with immune serum, the degree of inhibition of merozoite invasion, measured as a reduced parasitemia, varied from donor to donor, depending on the level of inhibitory antibody present. The more inhibitory serums gave significant (> 30 percent) inhibition at a serum titer of 1:16, the lowest level tested; the least potent serums gave little or no inhibition, even when used at 1:2 dilution. None of the serums collected from residents of Khartoum, a hypoendemic area for malaria, gave a significant inhibition titer.

In addition to the blocking of merozoite invasion, most of the immune serums examined produced moderate to severe retardation of intracellular parasite development. Parasites that succeeded in entering erythrocytes in the presence of immune serum failed to develop at the same rate as parasites in parallel cultures containing nonimmune serum. The degree of retardation of development varied from serum to serum and was dependent on the amount used to supplement the cultures. At 48 hours, most of the parasites in nonimmune cultures were mature segmenting schizonts, with a few newly invaded rings from precocious schizonts (Fig. 1, A and B), whereas those in immune serum often failed to develop beyond the ring stage, appearing as atypical rings or young trophozoites devoid of hemozoin pigment (Fig. 1C). As the concentration of immune serum was serially reduced from 1:2 to 1:16, the degree of parasite retardation was likewise reduced. Once this phenomenon was established with 20 different serums, some were absorbed against schizont-infected erythrocytes. Table 1 contains data on two typical immune serums that show significant retardation of parasite development. The degree of retardation at any given serum concentration can be appreciably reduced by absorption of the immune serum with parasitized erythrocytes. These observations suggested that intracellular parasite retardation by immune serum may have been antibody-mediated. Accordingly, purified immunoglobulin G from a pool of immune serums and from serum S-81-U21 were tested for inhibitory and retardation activity. The immunoglobulin G from the serum pool gave 70 percent inhibition at 6.5 mg/ml and that from S-81-U21 gave 68 percent inhibition at 8 mg/ml. Both purified immunoglobulin fractions retarded parasite development but to a lesser degree than whole serum did. However, since serum from malarious regions is often hypergammaglobulinemic it would be difficult to estimate

Table 1. Highly synchronized schizonts of *Plasmodium falciparum* were allowed to shed merozoites and develop in dilutions of nonimmune pooled human serum (NHS) and in two samples of immune serum. Serum S-81-U21 was obtained from the umbilical cord of a newborn whose mother had experienced symptoms of malaria during the final trimester of pregnancy. The concentration of the NHS did not appreciably affect the final parasitemia or the distribution of parasite stages. When NHS was absorbed with trophozoite- and schizont-infected erythrocytes (NHS-A) there was a slight retardation of parasite development, probably because of the removal of essential nutrients during absorption. As the concentration of immune serum was decreased the final parasitemia was increased, and the amount of retardation of parasite development was reduced. Absorption of the immune serums with infected erythrocytes removed significant amounts of inhibitory factors from these serums. (S-81-14-A and S-81-U21-A are S-81-14 absorbed and S-81-21U absorbed, respectively.) During absorption many segmenters released merozoites, and this probably accounts for the increase in total parasitemia after absorption.

Serum	Concentration (%)	Titer	Percent development by stages			Parasitemia (%)
			Rings	Trophozoites	Schizonts	
NHS	50	1:2	1	20	79	9.6
NHS-A	50	1:2	12.5	25	62.5	8.6
NHS	25	1:4	0	18	82	8.0
NHS-A	25	1:4	3	26	71	9.4
NHS	12	1:8	0	19	81	9.2
NHS-A	12	1:8	2	17	81	8.6
NHS	6	1:16	3	13	84	9.3
NHS-A	6	1:16	2	30	68	10.0
S-81-14	50	1:2	No living parasites			0
S-81-14-A	50	1:2	34	66	0	4.0
S-81-14	25	1:4	88	10	2	1.7
S-81-14-A	25	1:4	4	61	35	7.5
S-81-14	12	1:8	17	80	3	5.0
S-81-14-A	12	1:8	0	44	56	8.0
S-81-14	6	1:16	3	88	9	6.6
S-81-14-A	6	1:16	1	46	53	14.0
S-81-U21	50	1:2	No living parasites			0
S-81-U21-A	50	1:2	62	30	8	5.1
S-81-U21	25	1:4	64	36	0	2.9
S-81-U21-A	25	1:4	0	57	43	8.6
S-81-U21	12	1:8	0	55	45	7.9
S-81-U21-A	12	1:8	4	30	66	8.7
S-81-U21	6	1:16	0	26	74	9.6
S-81-U21-A	6	1:16	0	32	68	9.8

how much purified immunoglobulin would have specific antimalarial antibody equivalent to that in a volume of whole serum. Immunoglobulins from nonimmune serum had no effect on parasite development.

Two explanations could account for the above observations. Invading merozoites may become coated with antibody before entering the erythrocytes, and once the merozoites are intracellular, the immunoglobulins may exert their retardation effects. Another possible mechanism may be that parasite antigens inserted into the erythrocyte membrane, perhaps to mediate the uptake of nutrients essential for parasite development, may be blocked by antibody, resulting in starvation and retardation of parasite development. The parasite antigens on the erythrocyte surface need not be functional as transport mediators for the antigen-antibody complexes to alter erythrocyte permeability. To test the first hypothesis we allowed merozoites to invade in the presence of an immune serum having an indirect fluorescent antibody (IFA) titer of 1:50,000. Parasites at different stages of development were incubated with this serum and subsequently examined with ultraviolet fluorescent microscopy and IFA techniques. We were unable to detect any intracellular antibody on newly invaded rings or on segmenting schizonts, except when the erythrocyte membranes were obviously damaged. We did note, however, that methanol-fixed trophozoite- and schizont-infected erythrocytes showed antibody bound to the erythrocyte membranes of infected but not of uninfected cells. We noted this surface fluorescence on both "knobby" and "knobless" clones of FCR_3/Gambian parasites (*12*). Nonimmune serum did not produce surface fluorescence. We thus concluded that antibody is not transported into the erythrocyte during invasion, but is probably sloughed from the merozoite along with the surface coat during the endocytotic process (*13*). Since methanol-fixed infected erythrocytes produce surface fluorescence, but uninfected cells do not, the immune serum must contain antibodies against antigens found on the surface of infected erythrocytes, but not of uninfected erythrocytes.

Table 2. Cultures of *Plasmodium falciparum* were synchronized to a 6-hour age differential. Schizonts so prepared were allowed to invade through nonimmune pooled human serum (NHS) or three immune serums, CT, RO, or RO absorbed trophozoite- and schizont-infected erythrocytes (RO-A). After invasion was completed, the medium was replaced and the parasites were allowed to develop in the serum they invaded through, or were switched to NHS or immune serum. Giemsa-stained thin films were made after 48 hours. Parasites that invaded through normal serum but were switched to immune serums for development were markedly retarded in their development, as were parasites that invaded through and developed in immune serum. The exception to this pattern was RO-A, which after absorption, still inhibited invasion, but development was no longer retarded.

Serum used during invasion/ cultivation	Percent development by stages		
	Rings	Trophozoites	Schizonts
NHS/NHS	1.6	34.4	64
NHS/CT	14.6	79.2	6.2
NHS/RO	6.3	75.9	17.8
NHS/RO-A	1.5	34.5	64
CT/CT	40.7	56.5	2.8
CT/NHS	6.8	70.9	22.3
RO/RO	5.7	69.7	24.6
RO/NHS	2.8	41.6	55.6
RO-A/RO-A	1	40	59
RO-A/NHS	3.5	31	56

The hypothesis that immune serum exerts its retardation effects on the surface of infected erythrocytes was tested by allowing merozoites to invade erythrocytes in the presence of either nonimmune or immune serum, after which some cultures were switched from immune to nonimmune serum, or from nonimmune to immune serum (Table 2). Generally, parasites that invaded through and were cultured in immune serum were severely retarded in their development. However, when these parasites were switched to nonimmune serum for the development phase, the degree of retardation was significantly lower. Parasites that invaded through nonimmune serum and were switched to immune serum were also retarded in their development. Absorption of immune serum against trophozoite-infected erythrocytes removed the retardation factors, so that parasites exposed to this absorbed serum developed normally; the nonabsorbed immune serum retarded parasite development.

Infected erythrocytes cultured in the presence of immune serums contained numerous disintegrating parasites—the crisis forms. It was more difficult to demonstrate segmenters having a reduced number of merozoites. Usually the parasites deteriorated before they became schizonts. However, when synchronized cultures grown to the trophozoite stage in nonimmune serum were switched to immune serum, most of the parasites developed slowly and eventually produced abnormal segmenters containing 8 to 10 merozoites instead of the usual 14 to 16. These crisis-form merozo-

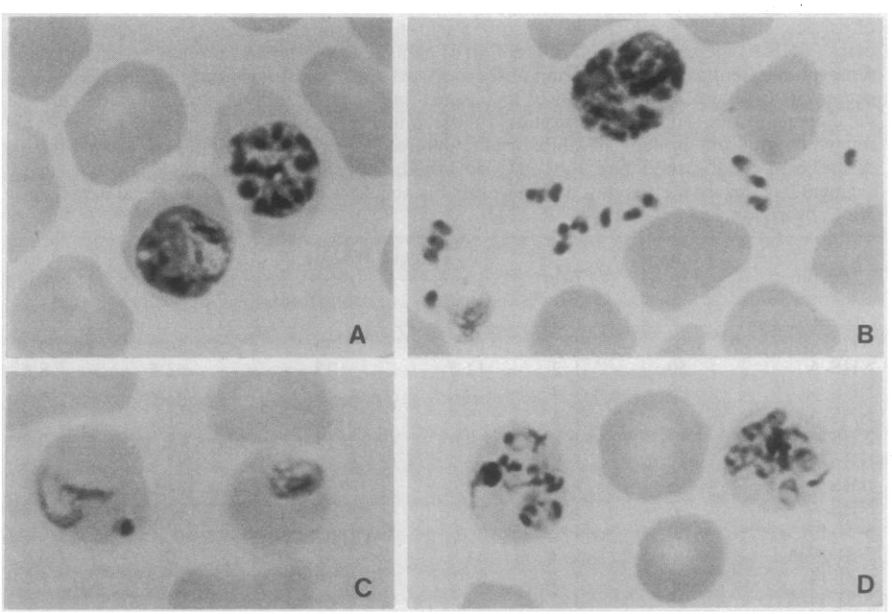

Fig 1. Highly synchronized cultures of *Plasmodium falciparum* were grown for 48 hours in nonimmune or immune serum. These photomicrographs of Giemsa-stained thin films demonstrate that parasites grown in nonimmune serum retained their synchrony, and the majority were mature and segmenting schizonts (A). The average number of merozoites was 14 to 16 as seen in (B), where a mature segmenter has just released 16 merozoites. Parasites from the same culture grown in serum S-81-14 (see Table 1) at 1:4 dilution failed to develop beyond the late ring–early trophozoite stage, were vacuolated, and stained poorly (C). When developing trophozoites were switched from nonimmune to immune serum many failed to produce segmenters (C). Others produced segmenters with enlarged merozoites, usually eight to ten in number, or were obviously deteriorating. In (D), the segmenter on the left contains two large merozoites and a degenerating residual mass, while the one on the right contains eight enlarged merozoites. All photomicrographs are ×5400.

ites were much larger than normal (Fig. 1, B and D). We also observed that if the synchronized parasites were exposed to the immune serum before segmentation, many failed to release their merozoites; thus reduction in the expected number of successful merozoite invasions may not be due entirely to merozoite-blocking antibody, but to a failure of schizonts to reach complete maturation.

Our results fully support the findings of Taliaferro and Taliaferro (*1*), who reported that the first indication of immunologic crisis in *P. brasilianum* infections was a loss of the characteristic parasite synchrony; this was accompanied by retardation of development and resulted in a reduced number of merozoites in the disintegrating intracellular parasites or segmenters. It would be difficult to observe crisis forms and intracellular deterioration of mature parasite stages in natural infections of *P. falciparum* because the trophozoite- and schizont-infected red cells are sequestered in capillaries and are rarely seen in peripheral circulation (*14*). Our findings are supported by a report that monoclonal antibodies to *P. falciparum* retard intracellular parasite development (*15*), but are at variance with several reports on the effects of immune serums on cultured parasites (*4*, *6*, *16*). Some of these investigations were conducted before the continuous cultivation of *P. falciparum* was achieved (*16*), and therefore the observations on dying cultures in vitro might not have given a true picture of the effects of immune serum. In other studies, current cultivation techniques were used with serums obtained from *Aotus trivirgatus* monkeys made refractory to infections with *P. falciparum* by repeated cycles of infection and drug treatment (*6*); these studies failed to demonstrate crisis forms and retardation of intracellular development of parasites, perhaps because the *Aotus* monkeys do not respond immunologically to *P. falciparum* infections as humans do.

We have demonstrated that immune serums collected from donors in the Sudan inhibit merozoite invasion into erythrocytes, as expected. In addition, these serums retard intracellular parasite development and lead to crisis forms and parasite disintegration. The inhibitory factors are apparently specifically acquired because serums from residents of Khartoum, who are of the same genetic stock as people in rural villages, are not inhibitory and do not retard parasite development. The most plausible explanation for the action of these immune serums is that they prevent the function of parasite antigens inserted into the erythrocyte membrane for the nutritional benefit of the developing parasite. Parasite antigens on the surface of infected erythrocytes have been described (*12*). The complexing of antibody to such antigens may block the uptake of essential nutrients and lead to retardation of parasite development. Since it has been generally accepted by investigators in malaria immunology that protective antibodies to malaria are directed against the merozoite, our results could lead to the discovery of protective antigens not associated with the merozoite.

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29 January 1982; revised 30 March 1982

HEL Cells: A New Human Erythroleukemia Cell Line with Spontaneous and Induced Globin Expression

Abstract. *A new human erythroleukemia cell line has been established. This line, designated HEL, is capable of spontaneous and induced globin synthesis, producing mainly Gγ and Aγ chains. Embryonic chains (ε, ζ) and α chains are detectable in very small amounts; β chains are undetectable. This line provides a new model system for studying aspects of erythroid cell differentiation and differential globin gene expression.*

Established cell lines are invaluable tools in cell biology. Murine hemopoietic cell lines, specifically the Friend erythroleukemia cells, have greatly expanded our knowledge regarding hemopoietic cell proliferation and differentiation and have been useful in studying gene expression. Recently, human hemopoietic cell lines established from patients with leukemia have become available (*1*); K-562 has been the only human cell line which expresses phenotypic markers of erythroid lineage (*2*) and displays spontaneous and induced globin synthesis (*3*).

We report here the establishment of a new human hemopoietic cell line which is capable of globin synthesis. This new cell line, designated HEL (for human erythroleukemia), was derived from the peripheral blood of a patient with Hodgkin's disease who later developed erythroleukemia. The patient received allogeneic bone marrow transplantation, but relapsed 7 months later. At the time of his relapse, Wright's Giemsa–stained blasts from peripheral blood and bone marrow resembled large abnormal proerythroblasts with basophilic agranular cytoplasm and showed a marked tendency for polyploidy. Their cytochemical characteristics (positive in periodic acid–Schiff reagent, acid phosphatase, and butyrase; negative in myeloperoxidase, chloroacetate esterase, and Sudan black B) were consistent with the diagnosis of erythroleukemia. Peripheral blood mononuclear cells from this patient at the time of relapse were used to initiate suspension cultures. Cells were cultured in

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