show similar effects. The most obvious of these is the Late Cretaceous, Coniacian to Campainian, which was characterized by tectonic reorganizations, warm climates, abundant carbonate sediments, and deep-sea cherts.

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Plasmodium falciparum Malaria: Band 3 as a Possible Receptor During Invasion of Human Erythrocytes

Abstract. *Human erythrocyte band 3, a major membrane-spanning protein, was purified and incorporated into liposomes. These liposomes, at nanomolar concentrations of protein, inhibited invasion of human erythrocytes in vitro by the malaria parasite* Plasmodium falciparum. *Liposomes containing human band 3 were ten times more effective in inhibiting invasion than those with pig band 3 and six times more effective than liposomes containing human erythrocyte glycophorin. Liposomes alone or liposomes containing erythrocyte glycolipids did not inhibit invasion. These results suggest that band 3 participates in the invasion process in a step involving a specific, high-affinity interaction between band 3 and some component of the parasite.*

Malaria remains a major public health problem in many areas of the world, with an estimated 150 million cases resulting in 2 million deaths each year (*1*). Victims are inoculated by mosquito bite with the sporozoite stage of the malarial parasite, and the sporozoites rapidly invade hepatic parenchymal cells and differentiate into merozoites. The merozoites are released into the circulation, where they invade erythrocytes. Infected erythrocytes soon rupture, releasing multiple merozoites that invade other erythrocytes, leading to chronic parisitemia. Invasion of erythrocytes is a multistep process that involves (i) attachment of merozoites to the erythrocyte membrane in a random orientation; (ii) reorientation of the attached merozoites such that the apical end of the parasite is opposed to the erythrocyte membrane; (iii) formation of a junction between the apical end of the merozoite and the erythrocyte membrane; and (iv) invagination of the erythrocyte membrane around the attached merozoite to form a vacuole inside the erythrocyte (*2*).

Several lines of evidence indicate that the invasion process requires specific interactions between the merozoite and the host erythrocyte (*3*). Erythrocyte membrane proteins glycophorins A, B, and C have been implicated as one of the attachment sites for *Plasmodium falciparum* (*3*), the species that causes the most virulent form of human malaria. Invasion of erythrocytes by *P. falciparum* in vitro has been reduced by genetic deficiency of glycophorins (*4*), digestion of glycophorin with trypsin or neuraminidase (*3*), and addition of isolated glycophorin to the assay medium (*5*). Friedman *et al.* (*6*) suggested that glycophorins A and B are involved in a relatively nonselective, charge-mediated attachment between merozoites and the red cell membrane, since various polyanions also inhibit invasion and since orosomucoid, a serum sialoglycoprotein unrelated to glycophorin, restores the invasion capacity of erythrocytes depleted of glycophorins (*6*).

Band 3, a major cell-surface protein in erythrocyte membrane, is a logical candidate to mediate specific red cell associations with malarial parasites. In support of this idea, Miller *et al.* (*7*) found that monoclonal antibody against rhesus monkey band 3 blocks invasion of rhesus erythrocytes by *Plasmodium knowlesi* parasites. We report here that human erythrocyte band 3 incorporated into liposomes is a potent inhibitor of invasion of human erythrocytes by *P. falciparum*, further supporting the hypothesis that band 3 participates in a high-affinity interaction with merozoite surface components.

Band 3 was purified from human erythrocyte ghosts by selective extraction of peripheral membrane proteins, followed by solubilization of membranes with nonionic detergent and fractionation of the detergent extract by ion-exchange chromatography (Fig. 1). Band 3 is not soluble in the absence of deter-

gent and therefore cannot be added directly to invasion assays. However, it is possible to incorporate band 3 into artificial phospholipid bilayer vesicles (liposomes), which maintain the protein in a native state in the absence of detergent. Band 3 and glycophorin (isolated by the same procedure as for band 3) were incorporated into liposomes by the procedure of Yu and Branton (*9*), which yields single-walled liposomes 40 to 80 nm in diameter. Band 3 reconstituted by this method forms intramembrane particles indistinguishable from particles in native membranes (*8*).

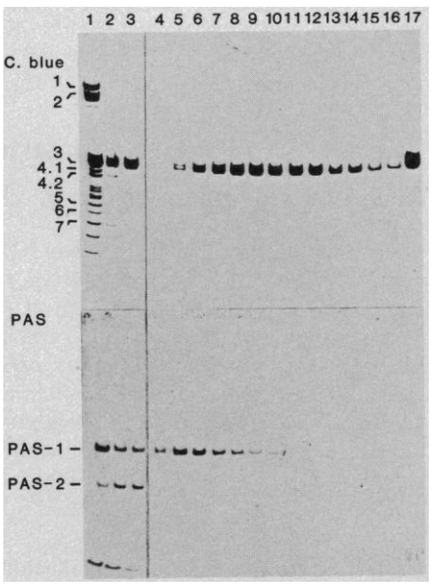

Fig. 1. Purification of human erythrocyte band 3 and incorporation of band 3 into liposomes. Potassium iodide–extracted inside-out membrane vesicles prepared from 40 ml of human erythrocyte ghosts (*8*) were solubilized at 4°C in 50 ml of 0.5 percent (by volume) Triton X-100, 10 m*M* sodium phosphate, 1 m*M* sodium EDTA, 1 m*M* dithiothreitol, and 1 m*M* NaN_3 (*p*H 7.5). The 100,000*g* supernatant of this extract was applied to a 15-ml column of DEAE-cellulose equilibrated in solubilization buffer. The column was washed with two bed volumes of buffer and eluted with a linear gradient of NaCl (0.1 to 0.3*M*) dissolved in solubilization buffer. Fractions were monitored for protein and analyzed by SDS electrophoresis (*8*). Fractions containing glycophorin (fractions 4 and 5) or band 3 (fractions 11 to 15) were pooled and the proteins were incorporated into liposomes by removal of Triton X-100 on Bio beads followed by addition of egg lecithin and sodium cholate and extensive dialysis (*9*). The liposomes were resuspended in phosphate-buffered saline. Samples were analyzed by SDS–polyacrylamide gel electrophoresis and the gels were stained with Coomassie blue or PAS to visualize sialoglycoproteins. Lane 1, erythrocyte ghosts; lane 2, potassium iodide–extracted vesicles; lane 3, Triton X-100 extract; lanes 4 to 16, fractions from DEAE chromatography; and lane 17, liposomes containing band 3.

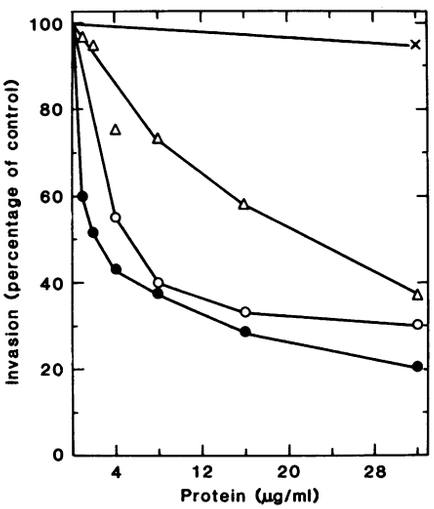

Fig. 2. Effect on reinvasion of erythrocytes by *P. falciparum* malarial parasites of increasing concentrations of liposomes containing human erythrocyte band 3 (●), human erythrocyte glycophorin (○), pig erythrocyte band 3 (△), or no protein (x). The Camp strain of *P. falciparum* was cultured (*10*) in RPMI 1640 medium supplemented with 10 percent human A^+ serum (heat-inactivated at 56°C for 30 minutes). Synchronized cultures were obtained by treating asynchronous cultures with sorbitol (*11*). The schizonts were isolated free of ring forms (immature parasites) by layering the culture mixture over 4 ml of a 15 percent metrizamide gradient and the gradient was centrifuged at 300*g* and 20°C for 15 minutes. After centrifugation the dark band at the metrizamide-medium interphase (the layer of schizonts) was aspirated in a laminar flow hood, washed once in 10 volumes of RPMI 1640 complete medium, and diluted to a concentration of 2.5×10^7 schizonts per milliliter. Reinvasion by merozoites was assayed in 10-mm microtiter wells. Washed human O^+ erythrocytes were suspended to a 4 percent hematocrit in RPMI 1640 complete medium containing 10 percent human A^+ serum. To each well was added 25 μl of the 4 percent cell suspension, 25 μl of phosphate-buffered saline, various concentrations of liposomes containing human or pig band 3 protein glycophorin, or liposomes at equivalent dilution but lacking protein. Schizonts (50 μl; 2.5×10^7 per milliliter) were added to each well, making the final erythrocyte concentration 1 percent and the initial parasitemia between 1.0 and 2 percent. The mixtures were mixed thoroughly and incubated for 3 hours in air with 6 percent O_2 and 5 percent CO_2. During the 3-hour incubation the contents of the wells were resuspended for 15 seconds every 30 minutes. After 3 hours of incubation most of the medium in each well was carefully removed and smears were made of the cells. The smears were air-dried, flushed with anhydrous methanol, dried again, and stained with Giemsa stain, and the number of intracellular ring stages of the parasites were counted per 10,000 erythrocytes. Data are expressed as the percentage of invasion relative to control wells with buffer added and are means of triplicate determinations. The level of control invasion in this experiment was 774 ring stages per 10,000 erythrocytes.

Invasion of human erythrocytes with cultures of *P. falciparum* (Camp strain, Vietnam) (*9*) synchronized by sorbitol (*10*) was measured (Fig. 2). Human erythrocyte band 3 inhibited invasion 50 percent at 2 μg per milliliter of protein, which is about 11 n*M* band 3 dimer. Invasion was inhibited 80 percent at the highest concentration of band 3 tested. Equivalent inhibition by human band 3 was measured with 12 different preparations of liposomes with band 3 from different donors.

Several controls demonstrated the specificity of the inhibition by human band 3. First, liposomes without protein, prepared under identical conditions and at equivalent dilutions, gave little inhibition. Second, liposomes prepared with pig erythrocyte band 3 were ten times less effective, with 50 percent inhibition at 20 μg per milliliter of protein (110 n*M* band 3 dimer). Finally, liposomes containing erythrocyte glycolipid did not inhibit invasion. Thus inhibition required band 3 in addition to lipid, and human band 3 was much more effective than a closely related band 3 from a species resistant to *P. falciparum* marlaria. Band 3 in red cells has two domains: an externally oriented domain that is relatively resistant to protease and an internal region facing the cytoplasm that is released by limited proteolysis (*11*). The external domain of band 3 in liposomes was responsible for inhibition of invasion, since unaltered inhibition was obtained with mildly proteolysed band 3 liposomes that had lost any exposed cytoplasmic domain.

Liposomes containing human glycophorin also blocked invasion, but less actively than those containing human band 3 (Fig. 2). Fifty percent inhibition of invasion by glycophorin required 5 μg/ml (80 n*M*), a concentration about seven times higher than that for human band 3. The activity of glycophorin in blocking invasion, although weaker than that of band 3, was comparable to or greater than that reported previously (*5*).

These experiments confirm the ability of glycophorin to inhibit invasion of erythrocytes by *P. falciparum* and demonstrate that band 3 incorporated into liposomes also blocks invasion at substantially lower concentrations than those of glycophorin. Analysis on sodium dodecyl sulfate (SDS) gels stained with Coomassie blue or periodic acid–Schiff reagent (PAS) showed the band 3 in the liposomes to be 90 percent pure

(Fig. 1). Other membrane components, such as minor glycoproteins and macroglycolipids, probably also are present, and in principle could contribute to the inhibition. However, because band 3 is the major component and liposomes are effective at low concentrations, a reasonable working hypothesis is that band 3 is the active component in these liposomes. The precise mechanism of inhibition by band 3 and glycophorin remains to be established. Explanations based on toxicity to parasites or alteration of erythrocyte membrane properties are unlikely, since controls with band 3 from a different species or with liposomes alone were much less effective. A plausible interpretation is that liposomes containing band 3 bind to surface sites on the parasites and thereby block their attachment to band 3 on the erythrocyte membrane.

Participation of band 3 in invasion is not surprising, since this cell-surface protein is present in 1 million copies per cell. Band 3 is the principal component of intramembrane particles visualized by freeze-fracture electron microscopy (*8*). Attachment of band 3 to merozoite surface components could explain the rearrangement of intramembrane particles into a ring surrounding a particle-free region that occurs at the junction between merozoite and erythrocyte membrane (*12*). Band 3 is attached on the cytoplasmic surface of the membrane to the spectrin-actin membrane cytoskeleton by linkage to ankyrin (*13*). Thus removal of band 3 from the particle-free zone (the site of merozoite entry) would also clear this region of the spectrin meshwork and allow penetration of the parasite.

Malarial parasites infect many vertebrate species, including reptiles, birds, and mammals. It is likely that different species share some fundamental features of the process of invasion. Band 3 has closely related homologues in these species and may represent a common receptor for all malarial strains. It is pertinent to note that band 3 has been implicated in invasion of rhesus monkey erythrocytes by *P. knowlesi* (*7*).

It will be important to identify the putative band 3 receptor of *P. falciparum* merozoites. Such a protein would be the logical target for vaccines against malaria.

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Trans-Acting Transcriptional Regulation of Human T-Cell Leukemia Virus Type III Long Terminal Repeat

Abstract. *Human T-cell leukemia virus type III (HTLV-III) was recently identified as the probable etiologic agent of the acquired immune deficiency syndrome (AIDS). Here it is shown that, in human T-cell lines infected with HTLV-III, gene expression directed by the long terminal repeat sequence of this virus is stimulated by more than two orders of magnitude compared to matched uninfected cells. The rate of transcription of the HTLV-III long terminal repeat is more than 1000 times that of the SV40 early promoter in one infected cell line. Thus, HTLV-III, like HTLV-I, HTLV-II, and the bovine leukemia virus, is characterized by* trans*-activation of transcription in infected cells. The efficiency of* trans*-activation in the case of HTLV-III may account, at least in part, for the virulent nature of HTLV-III infection.*

The human T-cell leukemia viruses (HTLV) are retroviruses associated with disorders of the $OKT4^+$ (helper) subset of T lymphocytes. HTLV type I (HTLV-I) is the probable etiologic agent of adult T-cell leukemia (*1*). HTLV type II (HTLV-II) is a rare isolate originally derived from a patient with a T-cell variant of hairy cell leukemia (*2*). HTLV-III is the probable etiologic agent of the acquired immune deficiency syndrome (AIDS), a disease characterized by depletion of the $OKT4^+$ cell population (*3*). In cells infected with HTLV-I or HTLV-II, the rate of transcription of heterologous genes directed by the viral long terminal repeat (LTR) sequences is greatly augmented (*4*). This phenomenon, called *trans*-acting transcriptional regulation, is also shared by bovine leukemia virus (BLV), a virus that appears to be structurally and functionally related to HTLV-I and -II (*5*). The genomes of HTLV-I, HTLV-II, and BLV have a long open reading frame (LOR) located between the *env* gene and the 3′ LTR

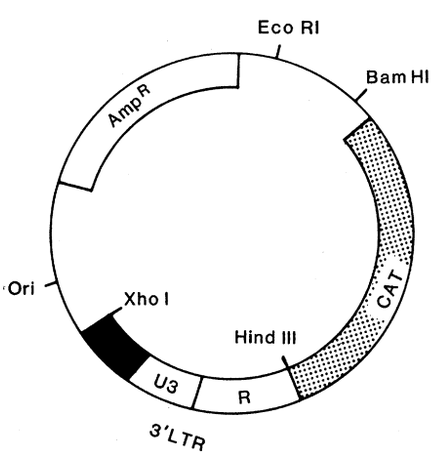

Fig. 1. Construction of pU3R-III plasmid. The diagram depicts the pU3R-III plasmid (Amp^R, ampicillin resistance gene; ori, bacterial origin of replication). The pU3R-III plasmid was constructed by isolating from the HTLV-III LTR cDNA clone C15 an Xho I–Hind III fragment containing the entire U3 and approximately 75 nucleotides of the R region (*11*). This fragment was inserted into the Xho-I–Hind III vector fragment of pSVIXCAT as previously described (*4*). Approximately 180 nucleotides of viral sequence 5′ to the LTR are included in the inserted fragment (solid black box). All recombinant DNA techniques were performed according to the enzyme manufacturer's specifications. Plasmids were purified by centrifugation in $CsCl_2$ gradients prior to transfection.