of these cells or their released lytic enzymes might account for permeability changes in these vessels.

In experiments where the cortical transplant was placed within the host cortex, it appears that new vessels formed at the interface were permanently altered and thus prevented the normal reconstitution of the BBB after trauma. Although the precise mechanism for CNS transplant permeability is not known, it seems likely that neovascularization and certain changes in capillary phenotypic expression are involved. In fact, fetal CNS graft survival may depend on an incomplete BBB. The early metabolic needs of young displaced neurons might best be met by an immersion in unidentified, blood-borne growth factors from the host. This permeability, however, would continue long after the cessation of transplant growth.

Because neural grafts can produce beneficial effects in certain situations, it remains to be determined if a deficient BBB has significant effects on either host or graft. These results suggest that CNS grafts could be directly affected by systemic administration of modulating compounds such as peptides or hormones. In addition, the transplanted tissue may be affected by both humoral and cellular constituents within the host's blood that characteristically are excluded from the brain.

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Identification and Isolation of a Variant Surface Glycoprotein from *Trypanosoma vivax*

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The protozoan *Trypanosoma vivax* is one of the most important agents of African trypanosomiasis, a disease that hinders the productive use of livestock in one-third of the African continent. *Trypanosoma vivax* is also present in the Caribbean and in South America, posing a threat to the livestock industries of the tropical and subtropical world. Much less is known of the biology of this trypanosome than of the better studied *T. brucei* and *T. congolense*. One of the variant surface glycoproteins (VSGs) of a West African stock of *T. vivax* was identified, purified, and partially characterized by the use of a combination of highly resolving techniques to maximize information from the relatively small amount of parasite material available. The molecular weight of the isolated protein (46,000) is smaller than that of VSGs from other species. As with *T. brucei* VSGs the protein from *T. vivax* is complexed with sugars and incorporates ^3H when living trypanosomes are incubated with [^3H]myristic acid, but the *T. vivax* molecule is more hydrophobic than the *T. brucei* molecule. The small size of the *T. vivax* VSG may have a bearing on the functional and evolutionary relationships of variant antigens in trypanosomes.

Trypanosoma (Duttonella) vivax, LIKE the other Salivarian trypanosomes, exhibits antigenic variation (*1*). The surface coat, the site of the variant surface glycoprotein (VSG), has traditionally been thought of as less compact in *T. vivax* than in other trypanosomes on morphological grounds (*2*). Furthermore *T. vivax* parasites are susceptible to the action of the bacterial toxin aerolysin to which *T. brucei* and *T. congolense* are resistant (*3*). Detailed studies of *T. vivax* have been hampered by the refractoriness of most laboratory hosts to this parasite of cattle as well as by the fragility of the organism and its apparent high rate of switching variable antigen types (*4*). For these reasons, it is difficult to obtain high parasitemias of organisms homogeneous for variable antigen types, which are required for biochemical investigation. We therefore used clones of a stock of *T. vivax* from West Africa that naturally infects rodents (*5*), and we sought techniques for VSG characterization and isolation that can be used with smaller numbers of trypanosomes (10^8 to 10^9) homogeneous for variable antigen types than are required for the purification of *T. brucei* VSGs (10^{11}) (*6*).

Most of our investigations were conducted with a clone, ILDat 1.2 (ILDat standing for ILRAD *Duttonella* antigen type), which was raised in irradiated C3H/He mice. We used two other similarly raised clones (ILDats 1.1 and 1.9) of the same serodeme (*4*) for comparison. Trypanosome populations were tested for homogeneity of variable antigen types by lysis tests with reference antisera (*4*). When samples of these populations were simultaneously run in two-dimensional polyacrylamide gels by use of the ISO-DALT system (*7*), only one protein train or cluster in the molecular weight range 41,000 to 50,000 differed among the clones in gels that had been stained with Coomassie brilliant blue. To determine

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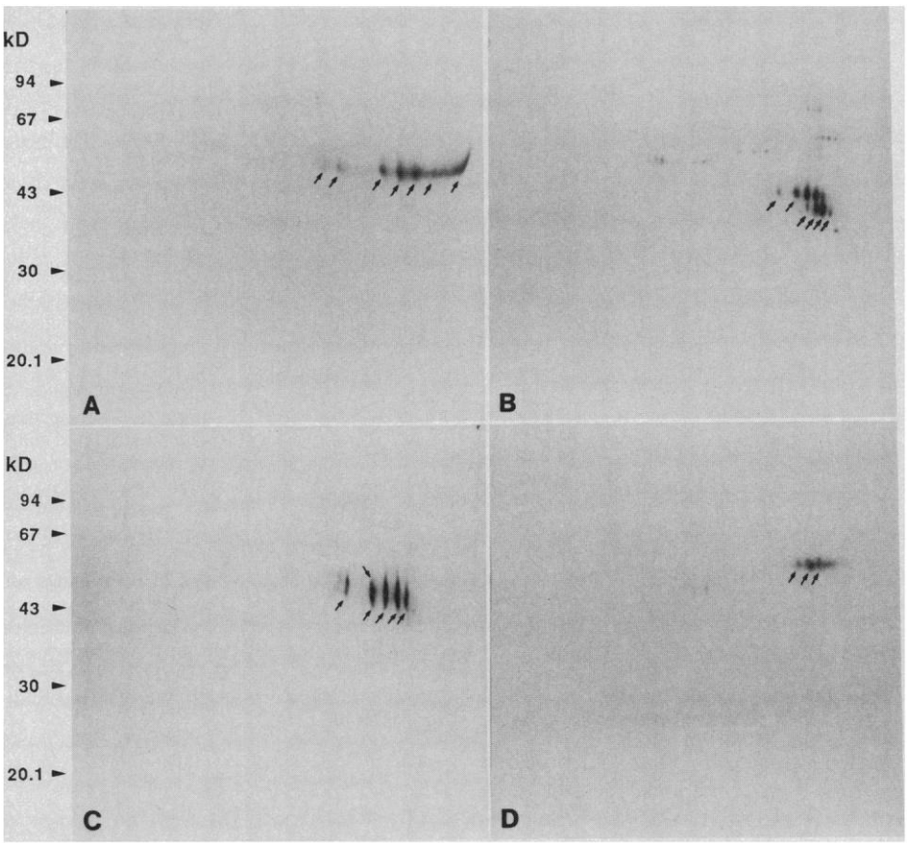

Fig. 1. Differences in the major biotinylated cell surface proteins of three clones of *T. vivax* and one of *T. brucei* as revealed by two-dimensional polyacrylamide gel electrophoresis. DE-52 purified trypanosomes (2×10^7 per milliliter) were biotinylated with 100 μg of sulfo-NHS-biotin (Pierce Chemical, Rockford, Illinois) in Dulbecco's phosphate-buffered saline, *p*H 7.2, for 10 minutes at 20°C, washed once by centrifugation, and solubilized at 10^9 organisms per milliliter in *p*H 9.5 solubilization buffer containing 9*M* urea, 2% 2-mercaptoethanol, 4% Nonidet P-40, and 2% ampholines at *p*H 9 to 11 (LKB, Bromma, Sweden). Samples (20 μl) were run using the ISO-DALT multiple 2-D system (*7, 8*). Ampholines in the first dimension were used in a 2:1 mixture of *p*H 3.5 to 10 and *p*H 4 to 6 types. The proteins were transferred to nitrocellulose by electrophoretic blotting (20 hours, 40 volts, 4°C). The blots were treated with streptavidin-biotinylated peroxidase complex (Amersham), and the biotinylated trypanosome proteins were detected by visualization in the presence of diaminobenzidine, H_2O_2, and Co^{2+} ions as recommended by the manufacturer. Molecular weight standards were run on a separate two-dimensional gel at the same time. All gel photographs are shown with the acid end to the left. (**A**) ILDat 1.2; (**B**) ILDat 1.1; (**C**) ILDat 1.9; and (**D**) *T. brucei* ILTat 1.3 (*9*).

whether these clone-specific proteins were exposed on the cell surface, we used cell surface biotinylation (*8*), since this technique, among the methods of labeling attempted, best preserved trypanosome integrity and motility. Examination of the two-dimensional protein maps after biotinylation of intact trypanosomes showed an extended charge train of spots representing the major ILDat 1.2 surface molecule at the basic edge of the gel (Fig. 1A). By comparison with molecular weight standards, this molecule had a molecular weight of 43,000 to 46,000. In contrast, ILDat 1.1 showed two sets of spots, one above the other (Fig. 1B), suggesting differences in glycosylation states of a protein with neutral sugars. The major ILDat 1.9 surface molecule (Fig. 1C) showed extended vertical streaks in the same region of the gel, the streaks showing differences in density. A similarly treated clone of *T. brucei*, ILTat 1.3 (ILTaT standing for *Trypanozoon* antigen type), incorporated biotin only in the VSG, previously determined (*9*) to have a molecular weight of 58,000 to 60,000, and one other molecule (Fig. 1D). The appearance of labeled proteins other than the VSG has been noted following various cell-surface labeling techniques with coated trypanosomes (*10*). This has been ascribed to the labeling of membrane proteins in small numbers of trypanosomes undergoing lysis during the labeling procedure (*10*). Whether the closeness of the packing of the major cell surface proteins in living *T. vivax* is sufficient to prevent the labeling of underlying membrane proteins is not known.

The molecular weights of the major cell surface proteins of the three *T. vivax* clones expressing different VSGs were more easily compared when the biotinylated proteins were separated in a single dimension on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). There was a major band of molecular weight 45,000 to 46,000 in ILDat 1.2, two components of almost equal intensity in ILDat 1.1 with molecular weights of 46,000 and 43,000, and one major (around 50,000 molecular weight) and one minor component (approximately 48,000) in ILDat 1.9. No change in the biotin-labeling pattern was observed when the labeling and subsequent processing steps were carried out in a mixture of protease inhibitors (*11*).

When ILDat 1.2 trypanosomes, solubilized directly after purification from mouse blood in 0.1% trifluoroacetic acid, were analyzed by reversed-phase high-performance liquid chromatography (HPLC) (*12*) a distinctive major peak was seen (Fig. 3A). The profile was generally similar to those

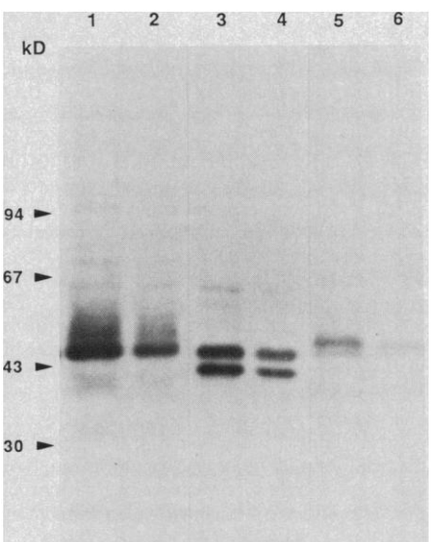

Fig. 2. Major biotinylated cell surface proteins of three clones of *T. vivax* as revealed by one-dimensional SDS-PAGE. Biotinylated trypanosomes were solubilized at 2.5×10^8 organisms per milliliter in buffer containing 2% SDS and 5% 2-mercaptoethanol and then boiled. Samples were run in SDS-PAGE (5% to 15% gradient), and the proteins were transferred to nitrocellulose and visualized as described. (Lanes 1 and 2) ILDat 1.2; (lanes 3 and 4) ILDat 1.1; and (lanes 5 and 6) ILDat 1.9. Lanes 1, 3, and 5 were loaded with 1.25×10^7 biotinylated trypanosomes; lanes 2, 4, and 6 were loaded with 1.25×10^6 biotinylated trypanosomes. No staining of unlabeled trypanosome proteins or molecular weight markers (Pharmacia, Uppsala, Sweden) run in the same gel occurred, although these were revealed by subsequent amido black staining of the nitrocellulose.

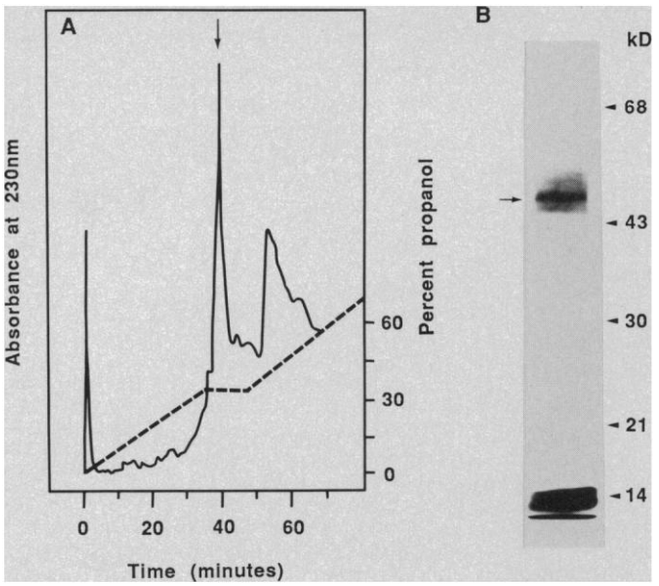

Fig. 3. (**A**) Typical absorbance profile of proteins of ILDat 1.2 solubilized in 0.1% trifluoroacetic acid (TFA) and fractionated by reversed-phase high-performance liquid chromatography. DE-52 purified ILDat 1.2 trypanosomes ($\sim 10^8$) were lysed directly in cold TFA and lyophilized. The lyophilizate was suspended in 100 μl of TFA and centrifuged at 14,000*g* for 5 minutes. The supernatant was injected onto a reversed-phase column (Altex RPSC C-4), and proteins were separated in a 0% to 60% 2-propanol gradient in 60 minutes on a high-performance chromatograph (Water Associates, Milford, Massachusetts). Eluate was monitored with an ultraviolet detector (Waters Model 481) at 230 nm. The fraction corresponding to the major peak (arrow) was dried, suspended in sample buffer, and run on one-dimensional SDS-PAGE. (**B**) The protein pattern of the major peak as revealed by SDS-PAGE. By comparison with molecular weight markers (Bio-Rad) the band indicated by the arrow had a molecular weight of 46,000.

obtained on analysis of *T. brucei* clones under the same conditions when the major peak was associated with the VSG. The material associated with the major peak in the ILDat 1.2 profile eluted at higher concentrations of organic solvent than with the *T. brucei* profile, indicating that the molecule was more hydrophobic than *T. brucei* VSGs. When the peak fraction was dried and assayed in one-dimensional SDS-PAGE, a single band of 46,000 to 47,000 molecular weight was seen (Fig. 3B).

The VSG molecule as it exists on the surface of other Salivarian trypanosomes so far examined contains a fatty acid at the COOH-terminus of the peptide chain (*13*). In the case of *T. brucei* the ester-linked fatty acid is dimyristyl glycerol, which is thought to serve as the membrane anchor for the VSG (*14*). Incubation of ILDat 1.2 trypanosomes in the presence of [^{3}H]myristic acid led to incorporation of label into a band of 46,000 molecular weight (Fig. 4), suggesting that the cell surface molecule we identified by biotinylation is equivalent to the VSG of *T. brucei* and has a complete COOH-terminus.

Rabbit antibody to the denatured 46,000 molecular weight protein excised from preparative SDS-PAGE gels was not lytic for ILDat 1.2 trypanosomes, as would be expected if most of the exposed epitopes of VSGs are conformationally dependent (*15*). However, the antigen recognized showed a cell surface distribution when frozen sections of the same trypanosomes were examined by immunocytochemistry with protein A–gold as the identifying conjugate. When

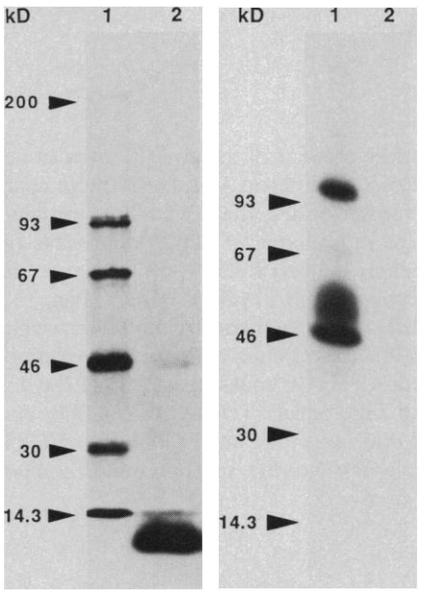

Fig. 4 (**left**). Fluorograph showing incorporation of ^{3}H into proteins of ILDat 1.2 (lane 2). ILDat 1.2 trypanosomes (2×10^7 per milliliter) were incubated at 20°C for 1 hour in RPMI-1640 (Gibco), *p*H 7.4, in the presence of 84 μCi of [^{3}H]myristic acid (Amersham) adsorbed to defatted bovine serum albumin (66 μg/ml). Washed, solubilized, and boiled trypanosomes were run in one-dimensional SDS-PAGE (5% to 15%) with [^{14}C]methylated protein molecular weight standards (Amersham) in lane 1. **Fig. 5** (**right**). Autoradiograph of ^{125}I-labeled surface proteins of ILDat 1.2 trypanosomes separated by SDS-PAGE after precipitation with Con A (lane 1) or with Con A in the presence of 0.2*M* α-methylmannopyranoside (lane 2) (*18*). By comparison with [^{14}C]methylated protein standards, the major 46,000 molecular weight surface molecule is specifically precipitated along with others. Similar results were obtained with RCA.

ILDat 1.2 trypanosomes were labeled in vivo with [^{3}H]myristate (or [^{35}S]methionine) and the labeled, infected whole blood was mixed with hot 4% SDS, the antibody precipitated a labeled molecule of 46,000 molecular weight after dilution of the sample in Triton X-100 to produce mixed micelles (*16*) in the presence of protease inhibitors (*11*). This result suggests that the apparent molecular weight of the ILDat 1.2 VSG is characteristic of the molecule in vivo.

Analysis of Formalin-fixed ILDat 1.2 by fluorescence-activated cell sorter showed that, in vitro, prior to fixation, trypanosomes could bind the fluorescein-conjugated lectins *Ricinus communis* agglutinin I (RCA), concanavalin A (Con A), and wheat germ agglutinin (WGA), but not soy bean agglutinin (SBA), or the agglutinin from peanut (PNA) at a concentration of 31.25 μg/ml. The binding of RCA and Con A were inhibitable by 0.2*M* concentrations of D-galactose and α-methylmannopyranoside, respectively, whereas *N*-acetylglucosamine only partially inhibited the binding of WGA. This binding of WGA, anomalously, was enhanced in the presence of sialic acid; the reason for enhanced binding in the presence of sialic acid is not yet understood. It is not possible to say unequivocally that the carbohydrates are exposed at the surface of *T. vivax* organisms in vivo, although this has been suggested by others (*17*). That the carbohydrates are borne on the VSG rather than, or as well as, on other cell surface molecules was shown by specific precipitation of the [^{3}H]myristate-labeled or iodinated (*18*) 46,000 molecular weight molecule with RCA or Con A (Fig. 5).

The accumulated data suggest that the VSG of *T. vivax* ILDat 1.2 is similar in general characteristics, except size, to VSGs previously studied and that the VSGs, at least of this serodeme of *T. vivax*, have molecular weights 8,000 to 14,000 lower than those reported for other species. In this report we have not distinguished between the membrane form of the VSG and the soluble form released on trypanosome disruption (*16, 19*). However, the incorporation of the tritiated label and the direct solubilization of purified trypanosomes in various highly denaturing reagents strongly suggest that the VSG molecule described above from ILDat 1.2 is the membrane form. It will be of interest to ascertain if this molecule can be converted, as in other trypanosomes (*16, 19*), to a soluble form.

Trypanosomes belonging to the genus *Duttonella*, in which the cyclical development in the vector is confined to the proboscis (*20*), are thought to be the most primitive Salivarian trypanosomes (*20*). Further-

more, this serodeme of *T. vivax* is said to lack minichromosomes, which carry telomeric VSG genes in *T. brucei* (*21*), and may therefore have alternative or simpler mechanisms for switching VSGs. The small size and hydrophobicity of the ILDat 1.2 VSG and the sensitivity of the membrane of this organism to aerolysin (*3*) suggest that studies on the packing of VSGs of *T. vivax*, their association with other cell surface molecules, and their mechanisms of release will be important in the elucidation of the evolution and function of variant antigens in trypanosomes.

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Synthesis of a Site-Specific DNA-Binding Peptide

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The Hin recombinase binds to specific sites on DNA and mediates a recombination event that results in DNA inversion. In order to define the DNA-binding domain of the Hin protein two peptides 31 and 52 amino acids long were synthesized. Even though the 31mer encompassed the sequence encoding the putative helix-coil-helix–binding domain, it was not sufficient for binding to the 26–base pair DNA crossover site. However, the 52mer specifically interacted with the site and also effectively inhibited the Hin-mediated recombination reaction. The 52mer bound effectively to both the 26–base pair complete site and to a 14–base pair "half site." Nuclease and chemical protection studies with the 52mer helped to define the DNA base pairs that contributed to the specificity of binding. The synthetic peptide provides opportunities for new approaches to the study of the nature of protein-DNA interaction.

MANY SEQUENCE-SPECIFIC DNA-binding proteins have been found to consist of two distinct structural and functional domains. One domain is responsible for recognizing and binding a DNA sequence, whereas the other may perform a second function such as binding to other proteins or catalyzing a specific reaction. The structural domains of some proteins, such as the *lac* repressor (*1, 2*), the λ repressor (*3, 4*), and the catabolite activator–binding protein (CAP) (*5*), have been physically separated by limited proteolytic digestion and subsequent purification of the products. It is difficult to prepare the large amounts of homogeneous peptide necessary for a variety of physical techniques including nuclear magnetic resonance (NMR) spectroscopy and x-ray crystallography, and it is difficult to modify the peptide with appropriate "reporter" groups, since it is derived biologically and contains only the 20 common amino acids. Using solid-phase peptide synthesis, we have prepared milligram quantities of a 52–amino acid peptide (52mer) that constitutes the DNA-binding domain of the Hin site–specific recombinase protein. This synthetic peptide has been used as a probe of the DNA-binding site, and its properties are compared to the DNA-binding properties of the intact Hin protein.

The Hin site–specific recombinase inverts a segment of DNA in order to change the expression of the flagellin genes of *Salmonella typhimurium* (*6*). Recombination occurs between two crossover sites designated *hix*L and *hix*R, in inverted repeat configuration, when they are on a supercoiled substrate (*7*). Each *hix* site is 26 bp long, has near twofold symmetry, and shares a 14-bp sequence with the other (see Fig. 1b). Hin is a member of a family of recombinases that complement one another and that includes Gin from phage Mu, Cin from phage P1, and Pin from the *e14* element of *Escherichia coli*. By comparing the crossover sites for all of these recombinases, the consensus sequence given in Fig. 1b has been determined (*8–10*).

The Hin family of recombinases is also related to the resolvase recombinase of transposon γδ. Hin and resolvase share 35% homology of amino acid sequence throughout their length (*11*). Resolvase has a two-domain structure (*12*); digestion with chymotrypsin cleaves it into two peptides. The COOH-terminal peptide binds to the resolvase DNA recognition site, and the NH_2-terminus mediates protein-protein interactions and presumably the recombination activities. The sequence homology implies that Hin should also fold into two domains, and its COOH-terminal domain should bind the *hix* site. We tested this hypothesis by synthesizing two peptides that correspond to the last 52 and 31 amino acids of Hin. The 52mer is homologous with the COOH-terminal fragment released from resolvase by chymotrypsin (Fig. 1a). The 31mer contains 21 residues that are homologous to the helix-turn-helix DNA–binding motif of known repressors and of resolvase. We show in this report that

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