Repetitive proteins from the flagellar cytoskeleton of African trypanosomes are diagnostically useful antigens

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SUMMARY

Trypanosome infection of mammalian hosts leads, within days, to a strong early response against a small, distinct number of parasite proteins. One of these proteins is the variable surface glycoprotein (VSG). Most of the others are apparently non-variable, intracellular trypanosome proteins. Two of these antigens I_2 and I_{17} are now characterized at the molecular level. Both exhibit a highly repetitive amino acid sequence organization, but they show no sequence similarity either to each other or to any other proteins known to date. Preliminary serological analyses indicate that both allow the early, sensitive and specific detection of infections with different species of trypanosomatids, making them interesting candidates for the development of diagnostic tools for trypanosomiasis detection.

Key words: *Trypanosoma brucei brucei*, *Trypanosoma congolense*, *Trypanosoma vivax*, cytoskeleton, repetitive protein, antigen, diagnosis.

INTRODUCTION

The strong immune response of a mammalian host infected with African trypanosomes is mainly directed against the variant surface glycoprotein, VSG (Cross, 1990) and is not protective (Seed & Sechelski, 1987; DeGee, Levine & Mansfield, 1988). This lack of protectivity is because of the high variability of the parasite's surface coat during the course of the infection. Much effort has been directed towards the search for invariant antigens both as tools for diagnosis and as potential targets for vaccine development. Such antigens are often high molecular weight proteins and associated with the flagellum or the cytoskeleton of the parasite (Lafaille *et al.* 1989; Ruiz *et al.* 1990; dos Santos *et al.* 1992). Müller *et al.* (1992) have identified 2 invariant, repetitive high molecular weight proteins associated with the cytoskeleton of the parasite which exhibited a high immunodiagnostic sensitivity. Time-course studies of the infection demonstrated that these 2 antigens were representatives of a whole group of high molecular weight (HMW) antigens which are all recognized very early in infection (Müller *et al.* 1993). Based on these findings, we have designed a screening strategy specifically aimed at identifying additional members of this group. A cDNA library from bloodstream *T. gambiense* was screened with affinity-purified antibodies against high molecular weight cytoskeleton proteins. The present study reports the identification of two new antigens, I_2 and I_{17}, by this strategy. Both exhibit a high repetitive sequence organization, and both are structural elements of the cytoskeleton.

MATERIALS AND METHODS

Trypanosomes

The trypanosomes used for cytoskeletal preparations, Western blot analysis, immunofluorescense and immunogold electron microscopy were all procyclic forms of *Trypanosoma brucei brucei* clone EATRO 427. They were grown in SDM-79 medium (Brun & Schönenberger, 1979) supplemented with haemin and 5% foetal calf serum. The cells were harvested during exponential growth at a cell density of about 5×10^6/ml.

Sera

Trypanosome cytoskeletons were prepared as described by Hemphill *et al.* (1991). Briefly, trypanosomes from 10 ml of culture were washed 3 times in MME (10 mM Mops/1 mM $MgCl_2$/0.2 mM EGTA, pH 6.9) and incubated in MME containing 0.5% Triton X-100 for 10 min on ice. After a centrifugation at 3000 g for 10 min the Triton X-100-insoluble cytoskeleton fraction was washed once in PBS (137 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4, pH 7.2) and resuspended in 100 ml of PBS. Rats were inoculated intraperitoneally with this cytoskeleton material either in complete (1st inoculation) or incomplete (2nd and 3rd inoculation) Freund's adjuvants. Blood was finally collected by cardiac puncture. Sera from experimentally infected mice were taken 14 days after an experimental infection with the LouTat 1 strain of *T. b. brucei* (DeGee *et al.* 1988). The bovine sera used for the evaluation

of the diagnostic sensitivity originated from cyclical or experimental infections of N'Dama and Zebu cattle with either *T. b. brucei, T. vivax* or *T. congolense*. The control sera originated from young (10 months) Zebu cattle. All bovine sera were kindly provided by Dr R. Mattioli, International Trypanotolerance Centre (ITC) in Banjul, The Gambia.

Lambda gt11 cDNA expression of T. b. gambiense

The cDNA expression library from bloodstream form *T. b. gambiense* (stock TREU 1285) was constructed in the cloning vector λgt11 as described (Barnes et al. 1989) and was kindly provided by Dr M. Selkirk (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London).

Immunoscreening of a cDNA library and construction of recombinant λ lysogen

The λgt11 expression library of *T. b. gambiense* was screened for clones corresponding to high molecular weight proteins. The antibody used for screening was prepared as follows. Whole procyclic trypanosome lysates (see below) were fractionated by SDS–PAGE and blotted onto nitrocellulose filters. After staining with 0·04 % Ponceau S in 10 % acetic acid, the high molecular weight region of the filter (> 180 kDa) was excised and used for affinity-purification of the antibodies from hyperimmune rat serum raised against trypanosome cytoskeletons (Müller et al. 1992).

Affinity purification of antibodies on fusion protein

Affinity purification of antibodies from rat anti-cytoskeleton hyperimmune serum on β-galactosidase fusion proteins was done as described by Müller et al. (1992). The affinity-purified antibodies were used at a dilution of 1:10 for Western blot analysis, immunofluorescence and immunogold electron microscopy.

Subcloning of λgt11 inserts

The cloning of recombinant λI_2 and λI_{17} cDNA inserts into Bluescript plasmid KS-plus (Stratagene) was performed as described by Rindisbacher et al. (1992).

Western blot analysis

The trypanosomal fractions used for the Western blot analysis were prepared as follows. For whole cell lysates $4·5 \times 10^8$ trypanosomes were washed once in MME and then lysed in 1 ml of sample buffer

(Laemmli, 1970). The cytoskeletal fraction was obtained by resuspending $4·5 \times 10^8$ cells in MME containing 0·5 % Triton X-100 and incubating them for 10 min on ice (Hemphill et al. 1991). For the preparation of the flagellar fraction, cytoskeletons were resuspended in 2 ml of 0·1 × MME containing 1 M NaCl (Schneider, Hemphill & Seebeck, 1988). After sonication for 15 s the suspension was kept on ice for 20 min. The cytoskeletons and the flagellar suspension as well as the supernatant of the cytoskeletons were all precipitated with 4:1 methanol/chloroform (Wessel & Flügge, 1984) to remove salts and detergent and the final protein pellets were solubilized in 1 ml of sample buffer. The Western blot analysis for the determination of the diagnostic sensitivity of I_2 and I_{17}/β-galactosidase fusion proteins was performed as described by Müller et al. (1992). Serum antibodies to β-galactosidase and other components of *E. coli* were previously removed by pre-adsorption of the bovine sera to a lysate of λgt11 lysogens from strain Y 1089 (Huynh, Young & Davis, 1985). The peroxidase-dependent colour reaction was performed using either 3,3′-diaminobenzidine tetrahydrochloride as described by Vogel et al. (1988) or Enhanced Chemoluminescence (ECL, Amersham) according to the manufacturer's specifications.

DNA sequencing and hybridization

Nested deletions of DNA fragments were prepared with exonuclease III double-stranded nested deletion kit (Pharmacia, Sweden); subclones were double-strand sequenced using the Sequenase DNA sequencing kit (United States Biochemical Corporation). For hybridization analysis, DNA was blotted onto nylon filters and was hybridized in 0·1 × SSC (15 mM NaCl, 1·5 mM Na₃citrate), 4 × Denharts, 0·08 % SDS, 80 mM sodium phosphate pH 6·5, 80 mg/ml herring sperm DNA at 65 °C for 14 h. The filters were washed 3 times for 10 min in 0·1 × SSC, 0·1 % SDS for 20 min at 65 °C. Both hybridizations were done using purified inserts of λI_2 and λI_{17} respectively.

Immunofluorescence

Trypanosomes were cultivated as described above. After 3 washes in PBS, cells (approximately 10^7/ml) were applied to cover-slips previously coated with 100 μg/ml polylysine and were allowed to settle for 1 h in a moist chamber at room temperature. Cover-slips were then rinsed twice in MME and treated with 2 % formaldehyde in MME for 15 min. The fixed trypanosomes were permeabilized in methanol at −20 °C for 10 min and subsequently rehydrated in PBS for 30 min. For the preparation of cytoskeletons, cells were extracted on the cover-slips with MME containing 0·5 % Triton X-100 for 5 min

at room temperature prior to fixation. After rehy-dration, cover-slips were incubated for 1 h in blocking buffer (PBS containing 100 mM L-lysine and 1 % BSA). For immunostaining, cover-slips were incubated for 45 min in a moist chamber at room temperature with affinity-purified antibody diluted 1:10 in blocking solution. After washing the cover-slips 6 times in PBS, FITC-conjugated rabbit anti-rat Ig (Dakopatts, Denmark; diluted 1:100 in blocking solution) was applied as described for the affinity-purified antibodies. After removing excess second antibody by washing 6 times in PBS, cover-slips were mounted on a slide using a mixture of gelvatol/glycerol in PBS as embedding medium (Lawson, 1983).

Immunogold electron microscopy

Washed trypanosomes were allowed to settle onto a Formvar carbon-coated grid for 1 h prior to ex-traction in MME containing 0·5 % Triton X-100 as described above. After rinsing the cover-slips in MME, the cytoskeletons were fixed in 3·7 % para-formaldehyde in MME, incubated in blocking buffer and exposed to the antibodies as described for immunofluorescence, with the modification that the second antibody was an affinity-purified goat anti-rat IgG coupled to 10 nm colloidal gold (Janssen, Beerse, Belgium), diluted 1:5 in 20 mM Tris–HCl, pH 8·2, containing 1 % BSA. After washing the grids 6 times for 5 min in PBS, they were fixed with 2 % glutaraldehyde in PBS for 20 min. Grids were then washed 6 times in H₂O, and negative staining was carried out in 3 changes of 1 % uranyl acetate, 20 s each. Finally, grids were air-dried and electron micrographs were taken with a Philips EM 300 microscope operating at 60 kV.

RESULTS

High molecular weight proteins associated with the cytoskeleton are antigens

When whole trypanosome extracts were anlaysed by immunostaining with infected bovine serum, a discrete banding pattern was observed (Fig. 1 A). Several distinct bands could be discerned in the 30–100 kDa region, and heavy staining was observed in the high molecular weight range, in agreement with earlier observations (Müller *et al.* 1992). Unexpectedly, the staining patterns obtained with whole cell lysates (Lane 1) and with purified cytoskeletons (Lane 2) were very similar, indicating that antibodies in the infected serum are mainly directed against cytoskeletal components. This no-tion is further supported by the observation that a qualitatively similar staining pattern was also ob-tained with hyperimmune rat serum raised against trypanosomal cytoskeletons (Fig. 1 B).
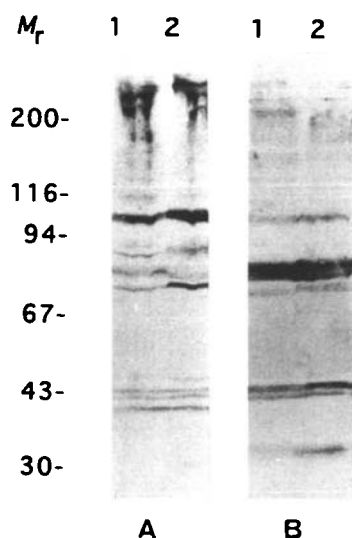

Fig. 1. Western blot analysis of whole trypanosomal cell extracts (Lane 1) and cytoskeletons (Lane 2) probed with (A) a bovine serum taken 14 days after infection and (B) with an anti-cytoskeleton rat hyperimmune serum. Molecular weight markers are given in kDa.
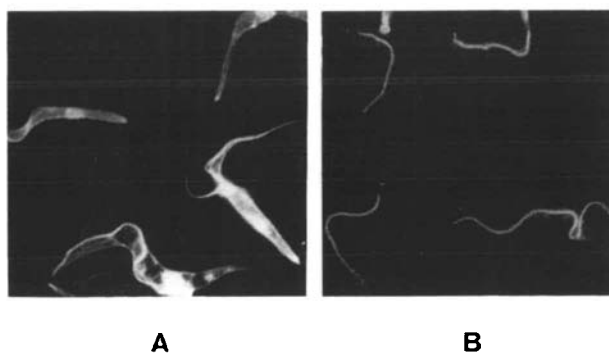

Fig. 2. Immunofluorescent staining of fixed trypanosomal cytoskeletons probed with (A) anti-cytoskeleton rat hyperimmune serum and (B) affinity-purified anti-HMW antibody.

In order to select antibodies with specificity against high molecular weight (HMW) proteins, the > 180 kDa region of gel-fractionated whole trypano-some extracts was used for affinity purification of antibody from hyperimmune anti-cytoskeleton serum. Immunofluorescence microscopy shows that hyperimmune serum against cytoskeletons reacts with almost the entire cell (Fig. 2 A) whereas affinity-purified HMW-specific antibodies predominantly stain the flagellum (Fig. 2 B).

Results presented in Figs 1 and 2 indicate that most of the host antibodies formed in the first 2 weeks of infection react with components of the cytoskeleton. Furthermore, many of the antibodies which are specific for HMW proteins react with components of the flagellum, suggesting that the flagellum is a source of strongly immunogenic antigens of high molecular weight.

A

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1    PHSVSRHNGQ  TQIAVGYAPS  FSELEABASI  VVLELGPFAR
41   BPVCSAVNID  SHSEKMDNLL  GVLLYAGSHF  SSRCIPISIV
81   RIPLCNCMR

90   BDLTKABELDBPVADTEVAEKBPTDSEVIPEKBIPDTBAASEQPA  (1)
135  ..............T..............................  (2)
180  ..............T..............................  (3)
225  ..............A..............................  (4)
270  ..............T..............................  (5)
315  ..............A..............................  (6)
360  ..............A..............................  (7)
405  ..............T..........

429  YIERDSLRSL  TMAEAKVSAB  KKAANPMRBI  VVKKSALTFA
469  WANLVIVSPV  HRRCSSNSAN  RLPFCPRAIN  GPYVWYPA*
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B

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1    LAVEALBELEBPQQVPAEAQPBAQ  PBGDI  (1)
30   ...............V........VA.....  (2)
59   ...............A.........L .....  (3)
87   ...............V........VA.....  (4)
116  ...............V........VA.....  (5)
145  ...............V........VA.....  (6)
174  ...............V........Q .....  (7)
202  ...............V........VA.....  (8)
231  ...............A........Q .....  (9)
259  ...............A........Q .....  (10)
287  ...............A........Q .....  (11)
315  ...............A........VA.....  (12)
344  ...............A........Q .....  (13)
372  ...............A........AA...   (14)

399  GBALVGLDVB  BFD*
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Fig. 3. Sequence analysis of the inserts in λI_2 and λI_{17}. (A) Deduced amino acid sequence from the λI_2 cDNA sequence. Between amino acids 90 and 428 the sequence consists of seven and a half highly conserved repeat units of 45 amino acids. One amino acid can be either alanine or threonine. (B) Amino acid sequence deduced from the λI_{17} cDNA sequence. The sequence consists of 14 highly conserved repeat units of either 29 or 28 amino acids. The first amino acid (leucine) of the given sequence does not belong to the repeat. At 2 positions within the repeat amino acid exchanges occur: from valine to alanine, and from glutamine to leucine or to valine plus alanine or to alanine plus alanine. The C-terminus of I_{17} consists of 13 unrepeated amino acids. In both panels, identical amino acids are indicated with a period and in-frame stops are marked by asterisks. The sequences have been deposited in the GenBank/EMBL data library under the accession numbers Z36280 (I_2) and Z36281 (I_{17}).

Fig. 4. Southern blot analysis of digested genomic *Trypanosoma brucei brucei* DNA. The DNA was hybridized with an I_2-specific probe (A) and with an I_{17}-specific probe (B). Size markers are *Hin*d III fragments of phage λ DNA.

Fig. 5. Western blot analysis of different trypanosomal fractions. Lane 1 contains whole trypanosomal cell extracts, Lane 2 contains the cytoskeletal fraction of trypanosomes, Lane 3 corresponds to the supernatant of the cytoskeletal fraction and Lane 4 contains a flagellar fraction of cytoskeletons. In (A) the filter was probed with affinity-purified anti-I_2/β-galactosidase fusion protein antibody and in (B) with affinity-purified anti-I_{17}/β-galactosidase fusion protein antibody. Molecular weight makers are given in kDa.

Screening of a T. b. gambiense *expression library with an affinity-purified antibody*

In order to identify genes for such HMW flagellar antigens, a λgt11 library of bloodstream *T. b. gambiense* (Barnes *et al.* 1989) was screened with the anti-HMW protein antibody. The phages recovered from this screening represented a number of different genes, two of them, I_2 and I_{17}, were further analysed in this study.

I_2 *and* I_{17} *both show repetitive sequence motifs*

The inserts of recombinant λI_2 and λI_{17} were subcloned into Bluescript plasmid KS-plus and were sequenced as described in the Materials and Methods section. The analysed fragment of I_2 has a length of 2004 bp. Its sequence has an in-frame stop codon at position 1518, followed by an untranslated str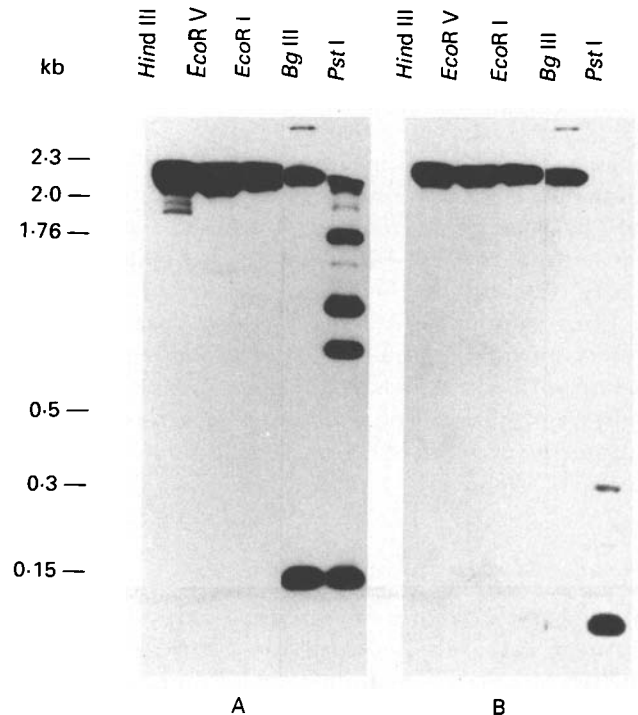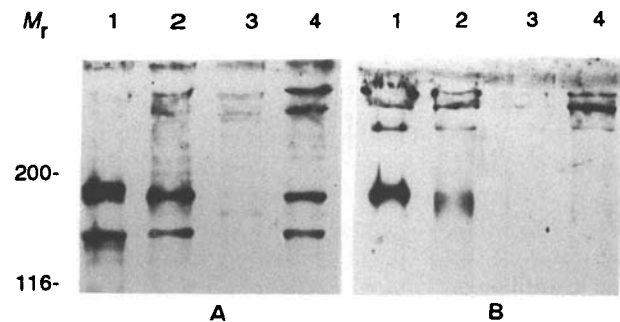etch and a poly-A tail (not shown). This suggests that the cloned cDNA fragment of I_2 represents the C-terminus of the I_2 gene. A 1017 bp stretch of the translated part (Fig. 3A) consists of a highly conserved 135 bp repeat. Only a single amino acid position of the repeat is not fully conserved and contains either alanine or threonine. The 3′ end of the coding sequence (300 bp) is not repetitive. The analysed fragment of I_{17} has a length of 1539 bp. The sequence has an in-frame stop codon at position
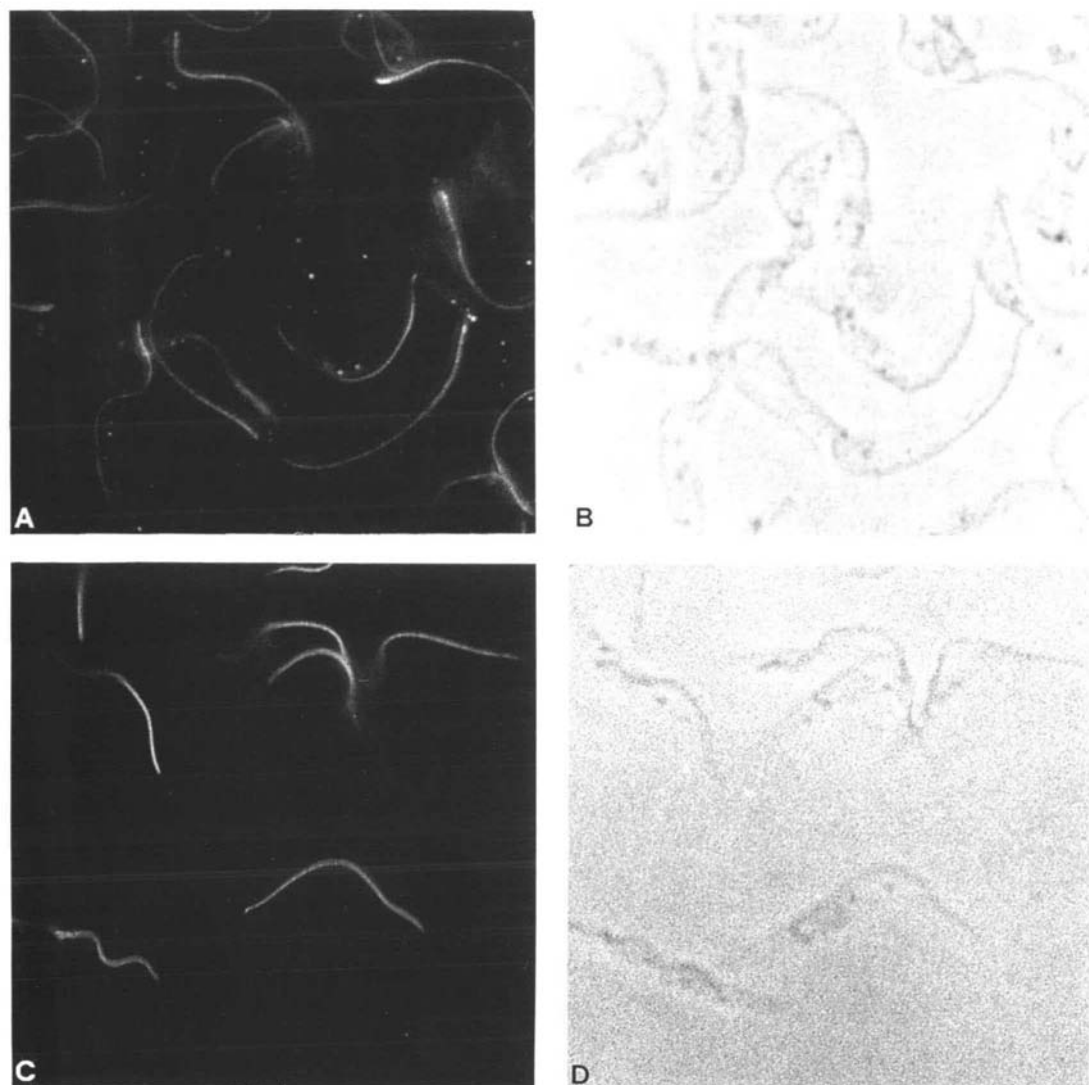
Fig. 6. Intracellular localization of I_2 and I_{17} by immunofluorescence microscopy (A and C). The cytoskeletal preparation of procyclic *Trypanosoma brucei brucei* was probed with affinity-purified anti-I_2/I_{17}/β-galactosidase fusion protein antibody. The corresponding phase-contrast micrographs are shown in B and D.

1233, followed by about 300 bp of untranslated region and a poly-A tail, which again indicates that the sequenced fragment represents the C-terminal part of the I_{17} gene. The derived amino acid sequence of I_{17} is shown in Fig. 3 B. Most of the I_{17} sequence (1179 pb) consists of a highly conserved repeat of 87 bp with only 3 positions not fully conserved. The C-terminus of I_{17} is formed by a short stretch (14 amino acids) of non-repetitive sequence. Database searching with both the I_2 and I_{17} amino acid sequences revealed no similarity with any known sequences.

Genomic organization of the I_2 and I_{17} gene

The cDNA sequence of I_2 and I_{17} showed that both genes are internally repetitive. The genomic organization of the genes of I_2 and I_{17} was subsequently determined by restriction mapping. Genomic DNA

of *T. b. brucei* was digested with restriction enzymes which either do or do not cut within the repeat sequence and was analysed by Southern blotting. Hybridization of genomic digests with an I_2-specific probe (Fig. 4 A) revealed that *Pst* I and *Bgl* I I, which both cut once within the I_2 repeat, generate a strongly hybridizing fragment, with the size of the repeat unit (135 bp). *Hin*dIII, *Eco* R V and *Eco* R I which do not cut within the repeat all generate large fragments (> 15 kDa). This observation indicates that I_2 consists of the conserved 135 bp repeat throughout most of its length. The additional fragments (1–2 kb) generated by *Pst* I may be indicative of occasional point mutations within the repeat.

Similarly, hybridization with the I_{17}-specific probe (Fig. 4 B) gives a strong signal in the upper molecular weight range (> 15 kb) for enzymes which do not cut in the repeat (*Hin*dIII, *Eco* R V, *Eco* R I and *Bgl*
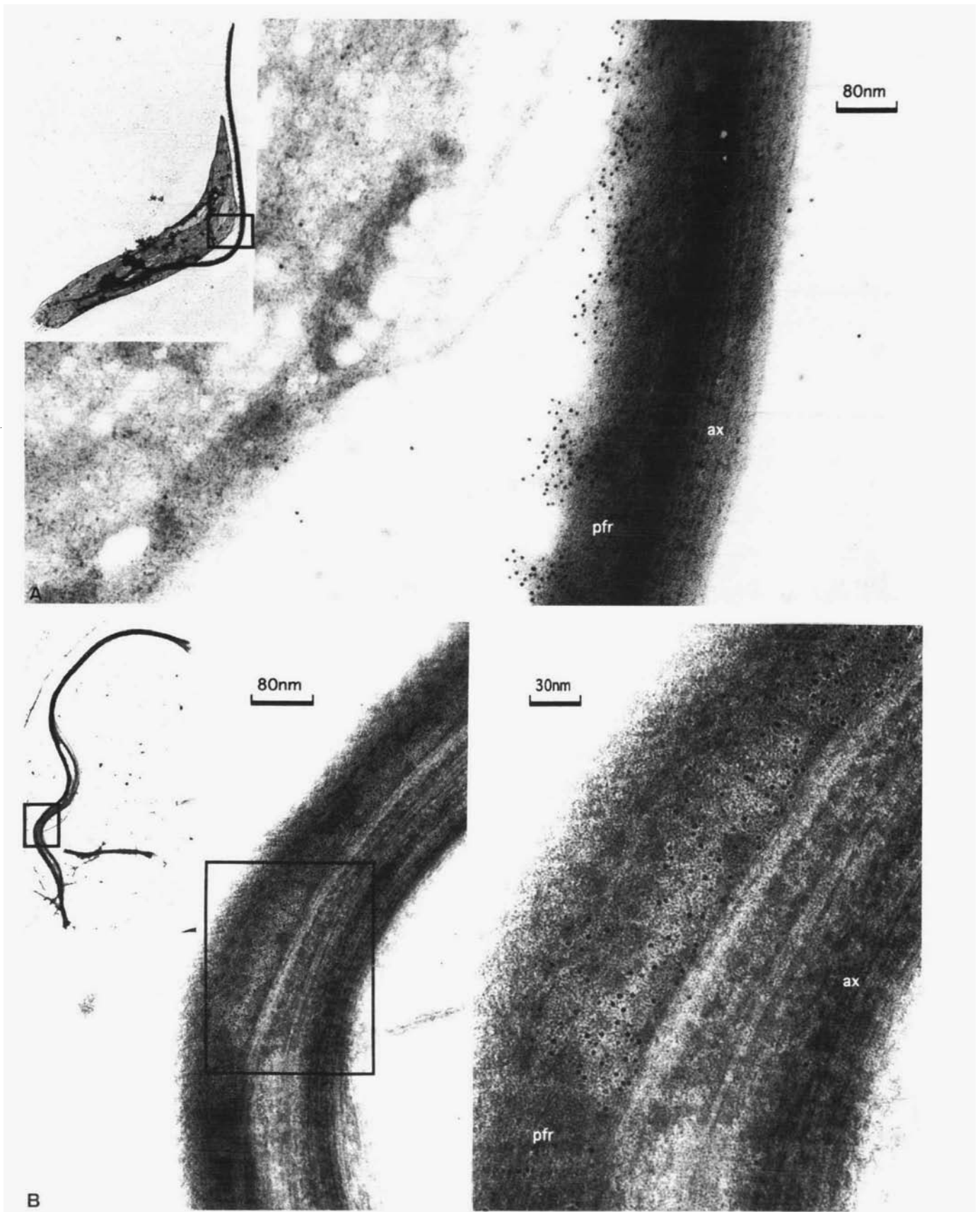
Fig. 7. Immunogold labelling of I_2 (A) and I_{17} (B) with negatively stained whole-mount cytoskeleton preparation probed with the corresponding affinity-purified antibodies (see Materials and Methods section). (A) Gold particles are present mainly along the paraflagellar rod (*pfr*) of the flagellum. (B) Gold particles cover the area between the paraflagellar rod and the axoneme (*ax*) of the flagellum.
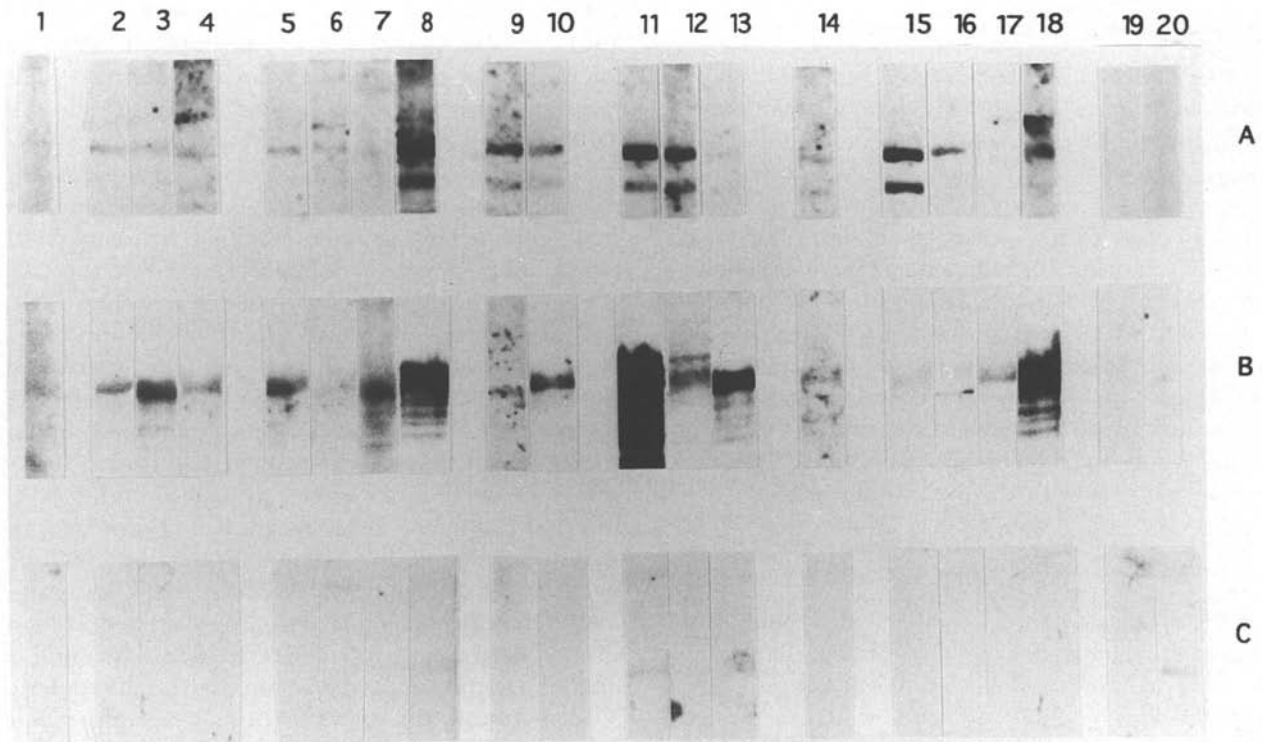
Fig. 8. Western blot analysis demonstrating the diagnostic sensitivity of I_2 and I_{17}. Protein extracts from *E. coli* lysogens of λI_2 (A), λI_{17} (B) and $\lambda gt11$ (C) were blotted and cut into strips. Each strip was then incubated with a bovine serum. The sera originated from cyclical infections by *Trypanosoma vivax* (Lanes 2–4), *T. congolense* (Lanes 5–8), *T. b. brucei* (Lanes 1, 9, 10), or from mixed infections by *T. b. brucei/T. vivax* (Lanes 11–13) or *T. b. brucei/T. congolense* (Lane 14) or from experimental infections with *T. congolense* (Lanes 15–18). Strips 19 and 20 were incubated with two different uninfected sera.

II) whereas *Pst* I, which cuts once within the repeat, generates a very strong band at about 90 bp. This correlates well with the size of the repeat defined by sequence analysis (87 bp). The additional two faint bands correspond to dimers and trimers of the repeat unit. This is in good agreement with the available sequence data which show that the *Pst* I restriction site is missing in three of the 14 repeats sequenced. In contrast to I_2, the I_{17} hybridization probe does not reveal additional bands, indicating that the whole I_{17} gene has a repeated structure.

I_2 and I_{17} are both high molecular weight proteins

The screening strategy used for gene isolation, as well as sequence and hybridization data predict that I_2 and I_{17} both are high molecular weight proteins. In order to confirm this prediction at the protein level, the following Western blot analysis was performed. Whole cells, cytoskeletons and flagella of trypanosomes were prepared (see Materials and Methods section) and fractionated by SDS–PAGE. After transfer to nitrocellulose, they were probed with affinity-purified antibodies against I_2 and I_{17}/β-galactosidase fusion proteins. Both antibodies revealed several distinct bands in the high molecular weight ($>$ 180 kDa) region. Both proteins are detected by immunostaining of whole cell extracts,

in Triton-insoluble cytoskeletons and in salt-extracted flagella (Fig. 5) indicating that both proteins are tightly associated to the flagellar cytoskeleton.

Localization of I_2 and I_{17}

Having established the tight association of I_2 and I_{17} with the flagellar cytoskeleton, an immunofluorescent staining of cytoskeletons and whole cells with affinity-purified anti-I_2 and anti-I_{17} antibodies was performed. The results of this staining are shown in Fig. 6. Both I_2 and I_{17} antibodies induce a bright fluorescence along the flagellum. As both antibodies stain the flagellum with a very similar pattern, a more detailed localization was required. This was achieved by immunogold electron microscopy (Fig. 7). Antibody against I_2 reacts with the paraflagellar rod (Schlaeppi, Deflorin & Seebeck, 1989) suggesting that I_2 is a component of this structure. In contrast, antibody against I_{17} specifically stains the interface between the paraflagellar rod and the axoneme. While immunogold electron microscopy thus confirms the biochemical and immunofluorescence data indicating a close association of I_2 and I_{17} with the flagellum, it further demonstrates that the detailed localization of the two proteins within the flagellum is quite distinct.

Immunodiagnostic potential of I_2 and I_{17}

In order to evaluate the potential of I_2 and I_{17} for diagnostic purposes, the respective β-galactosidase fusion proteins were used to analyse, by Western blotting, 18 sera from N'Dama and Zebu cattle with parasitologically confirmed infections with *T. b. brucei*, *T. congolense*, *T. vivax*, as single and as mixed infections. Also, two uninfected sera were included as controls. Fig. 8 demonstrates that 15/18 infected sera reacted with I^2 and 17/18 with I_{17} but none with the β-galactosidase control protein. All sera reacted with at least one of the two recombinant antigens. The serum which was taken 5 days after infection reacted with both recombinant antigens. No reactivity was seen with the 2 control sera.

DISCUSSION

In an earlier study we identified two trypanosomal antigens to which antibodies are produced early in an infection, MARP1 and GM-6 (Müller *et al.* 1992; Hemphill *et al.* 1992), as high molecular weight proteins both associated with the cytoskeleton. As both of them exhibited a high immunodiagnostic sensitivity (90%), we wanted to know if African trypanosomes contain such additional cytoskeletal antigens. Therefore we have designed a screening protocol for the identification of these antigens. The present study described two antigens isolated by this strategy, I_2 and I_{17}. Both are high molecular weight proteins (MW > 180 kDa), both are highly internally repetitive and consist predominantly of conserved repeat units of 45 and 29 amino acids, respectively. Thus I_2 and I_{17} share several important features with GM6 and MARP1, but all 4 proteins show neither amino acid sequence similarity nor immunological cross-reactivity (data not shown). I_2 and I_{17} are both located in the flagellum, though at different locations within this structure. The data presented in Fig. 8 indicate that I_2 and I_{17} might be of diagnostic interest in that they represent proteins which (i) are strongly immunogenic already in the early phase of infection, (ii) elicit an appreciable antibody response in all infected hosts, and (iii) are highly conserved between the African trypanosomatids (*T. brucei*, *T. congolense* or *T. vivax*).

The finding that these trypanosome antigens are highly repetitive is in line with what we already have described for two other early antigens, MARP-1 and GM6 (Müller *et al.* 1992, 1993). These observations contribute to the emerging overall picture that the host antibody response both in the African trypanosomiases as well as in South American Chagas disease is strongly directed against parasite proteins which have the common denominators of being (i) very large and (ii) highly internally repetitive with a very high degree of sequence conservation between the individual repeat units (Ibanez *et al.* 1988; Hoft *et al.*

1989; Lafaille *et al.* 1989; Duncan, Gay & Donelson, 1991; Pollevick *et al.* 1991; Burns *et al.* 1993). In addition, all these antigenic proteins are intracellular proteins, with the exception of SAPA, which is a GPI-anchored surface protein (Pollevick *et al.* 1991).

Several aspects of the molecular characteristics of these antigens are noteworthy. Already the mere fact that proteins consist of so many so well-conserved repeat units is peculiar. Highly repetitive proteins are not common in biology, and where they exist, e.g. spectrin in the erythrocyte, they exhibit a large extent of sequence variation between individual repeat units (Alcina *et al.* 1988). Conceptually, an internally repetitive organization of a protein might have evolved because the protein in question interacts with a repetitive substrate and is under evolutionary pressure to form many identical interactions with the substrate. This line of argument would be particularly cogent for MARP-1, which was originally identified as a microtubule-associated protein of trypanosomes (Schneider *et al.* 1988; Hemphill *et al.* 1992) and thus interacts with a lattice of regularly spaced tubulin dimers. However, although similar pressures should exist in other organisms, no comparable degree of repetitiveness is found elsewhere. Many microtubule-associated proteins from different organisms have been characterized and, where repetitive domains have been found, they consist of a small number of not very highly conserved repeat units (Lewis, Wang & Cowan, 1988).

These findings argue that it is not the biological function of the various internal trypanosome antigens which exert the pressure to build up highly repetitive proteins and to maintain the observed near-perfect sequence conservation. So what does? Is it conceivable that the high molecular weight repetitive proteins have been generated by the trypanosome as devices for liberating, upon cell destruction by host defence mechanisms, large amounts of identical, immunologically active peptides which stimulate the immune system? Since many of the infecting trypanosomes are invariably destroyed at the site of an infection, this concept would guarantee a rapid and effective stimulation of the host's immune system, some parts of which might be beneficial for the parasite. The concomitant stimulation of antibodies against the peptides would be of no consequence to the trypansome because the target proteins are safely sheltered inside the cell. A similar situation is the strong stimulation of the host's immune response by the variant surface coat (VSG) which is the most prominent external antigen of the trypanosome (Cross, 1990). Here a different VSG-type population of parasites is already growing when the response against the former one is fully raised. Thus the parasite, after having stimulated a response against itself, can survive without any disadvantage.

While we clearly need to learn much more about the possible roles of these repetitive proteins in the course of an infection, the available data demonstrate that they could play a most useful role, in a more practical sense, as diagnostic reagents.

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