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Identification of Immunogenic and Antibody-Binding Membrane Proteins of Pathogenic *Trichomonas vaginalis*

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Characterization of immunogenic *Trichomonas vaginalis* membrane proteins was accomplished by using extrinsically and intrinsically labeled organisms and a highly sensitive and specific radioimmunoprecipitation procedure. Intact motile trichomonads were compared with detergent extracts as a source of antigen in radioimmunoprecipitation experiments. Approximately 20 proteins accessible to antibody were identified and ranged in molecular weight from 200,000 to 20,000. Localization on the parasite surface of the highly immunogenic membrane proteins was attempted by using, as the indicator system, formaldehyde-fixed protein A-bearing *Staphylococcus aureus* pretreated with the various antiserum reagents and incubated with live, motile parasites. Also, indirect immunofluorescence with fluorescein isothiocyanate–anti-rabbit immunoglobulin was also employed after incubation of organisms with either control serum or antiserum from immunized rabbits or after treatment of trichomonads with the immunoglobulin G fraction from each respective serum. No immunoglobulin G antibody appeared to be directed at the anterior trichomonal flagella or the posterior axostyle, whereas strong fluorescence was detected throughout the rest of the *T. vaginalis* surface. The biological significance of these data is discussed.

Trichomonal vaginitis is a sexually transmitted disease responsible for significant morbidity in both men and women. In symptomatic females, the disease is characterized by pronounced inflammation of the vagina, foul-smelling vaginal discharge, severe discomfort, and tissue cytopathology (10, 13, 18, 20). Although most men appear to be asymptomatic, disease manifestations are increasingly being documented (19, 20). The causative agent, *Trichomonas vaginalis*, is the most frequently acquired protozoan infestation; however, few data are available on key areas of this host-parasite interaction which would yield information on possible mechanisms of disease pathogenesis.

Current clinical diagnosis of trichomoniasis is tedious, time consuming, highly inadequate, and based on microscopic detection of the parasite (10, 20). These limitations in diagnosis exacerbate already limited medical care in rural health clinics in our country and the world. Thus, basic research is necessary to address relevant issues such as development of sensitive, accurate serological assays for screening of symptomatic as well as asymptomatic patients and perhaps monitoring disease progression. The development of strategies to identify potential vaccinogen candidates is equally important and necessary.

The emergence of trichomoniasis as a major sexually transmitted disease (10, 13, 18, 20), therefore, has necessitated the need for research directed toward identification of possible virulence factors. The characterization of the surface of *T. vaginalis* has not been accomplished. Furthermore, the use of conventional immunological methods has failed to identify specific virulence determinants or antigens (10, 11, 21, 22). Because one of our aims is to assess host immunological responses to parasite antigens, the nature of antibody responses from immunized or infected experimental animals directed toward immunogenic parasite proteins was recently demonstrated (1). In this report, numerous highly immunogenic proteins which are exposed on the membranes of intact *T. vaginalis* and reactive with antibody while the membrane proteins are in their native state are identified. The importance of these data is discussed.

MATERIALS AND METHODS

Growth and radiolabeling of *T. vaginalis* organisms. *T. vaginalis* 286 (16) was a generous gift from Miklos Müller, Rockefeller University, New York, N.Y. Parasites were maintained in vitro in an air atmosphere as described elsewhere (17) with Diamond Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-maltose medium (pH 6.2) supplemented

with 10% heat-inactivated horse serum (Kansas City Biologicals, Inc., Lenexa, Kans.). Only trichomonads at the late-log stage (24 h) of growth (17) were utilized for experiments, and they represented a density of 2.5×10^6 to 5.0×10^6 actively motile parasites per ml.

Intrinsic radiolabeling with [^{35}S]methionine (specific activity, 1,500 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) has been previously described (1, 3, 17). Pellets containing 1.4×10^7 to 3.5×10^7 trichomonads were frozen at −70°C until use or employed immediately for the radioimmunoprecipitation (RIP) assay with whole cells as the antigen as described below. Electrophoretic protein patterns of frozen trichomonal pellets were identical to those of freshly harvested, radiolabeled organisms.

Specific radioiodination of membrane proteins was accomplished by standard chloramine-T labeling techniques (5). One hundred milliliters containing 2.5×10^8 motile organisms was washed at least three times in phosphate-buffered saline (PBS) to remove serum proteins contaminating trichomonal surfaces (17). Trichomonads were suspended to 1 ml in PBS followed by the sequential addition of 3 mCi of Na^{125}I (Amersham) and 100 μl of 0.4% chloramine-T (Sigma Chemical Co., St. Louis, Mo.). After a 5-min incubation at room temperature with constant gentle stirring, 100 μl of 0.4% $NaHSO_3$ was added to terminate the reaction. Trichomonads remained motile under these experimental conditions and were washed at least three times further with phosphate-buffered NaI (same as PBS, except NaI was substituted for NaCl) before aliquoting for freezing at −70°C or immediate use in the RIP with intact, motile organisms.

Serum reagents. Prebled control serum and antiserum against *T. vaginalis* strain 286 were generated in New Zealand white rabbits injected subcutaneously and intramuscularly with a mixture of 0.5 ml of trichomonal suspension containing 2.5×10^7 organisms and 0.5 ml of Freund complete adjuvant (GIBCO Laboratories, Grand Island, N.Y.). Booster injections were performed 2 weeks later with equal amounts of organisms in Freund incomplete adjuvant. Parasites were extensively washed three times in PBS to remove contaminating medium macromolecules adsorbed onto parasite surfaces (17). Rabbits were bled 14 days after booster immunization, and the sera were stored at −70°C until use. Adsorbed antiserum was generated by suspension of 1.7×10^7 trichomonads washed three times in PBS in 0.5 ml of antiserum, and the incubation was continued for 30 min at 37°C. The serum was removed after pelleting of the parasites, and the process was repeated 10 times. No lysis of organisms was detected based on cell counts with an improved Neubauer counting chamber. Alternate immunization protocols such as intravenous injection of formaldehyde-fixed organisms before or after the booster mentioned above neither modified nor amplified the antibody response as analyzed with these experimental procedures.

RIP assay. Zwittergent 3-12 (Z3-12) (Calbiochem-Behring Corp., La Jolla, Calif.) extracts of [^{35}S]methionine- or Na^{125}I-labeled *T. vaginalis* proteins were used as a source of antigen in the reaction mixture (1). One hundred microliters of a Z3-12–solubilized, radiolabeled preparation of strain 286 was mixed with 50 μl of serum reagent. After incubation at 4°C overnight, a 100-μl volume of protein A-bearing *Staphylococcus aureus* (12) was added to bind the antibody-antigen complexes. After extensive washing of the protein A-bearing *S. aureus* radiolabeled antigen(s) were released by suspending pelleted protein A-bearing *S. aureus* organisms in 100 μl of electrophoresis dissolving buffer (3, 17) and boiling for three min. The protein A-bearing *S. aureus* cells were then pelleted, and the supernatant was electrophoresed as described below for fluorographic detection of immunogenic trichomonal proteins.

The analysis of exposed membrane proteins on intact, motile *T. vaginalis* accessible to antibody by RIP was performed immediately after intrinsic or extrinsic radiolabeling of parasites proteins. This procedure has been recently and successfully employed for a bacterial model system (7, 8) and has been defined as a whole cell (WC) RIP. Approximately 1.25×10^8 trichomonads were pelleted after intrinsic labeling with [^{35}S]methionine (1, 17) or chloramine-T-mediated radioiodination and suspended with 100 μl of appropriate heat-inactivated control serum or heat-inactivated antiserum reagent. The suspension was incubated for 120 min at 37°C with occasional gentle stirring followed by washing of the trichomonads no less than twice in PBS. *T. vaginalis* organisms were then suspended in 200 μl of NET (150 mM NaCl, 5 mM EDTA, 50 mM Tris-hydrochloride, pH 7.2) buffer and solubilized by the addition of 25 μl of 10% Z3-12 detergent (4, 6). The preparation was gently homogenized, and an additional 200 μl of NET buffer was added. The extract was then centrifuged at 35,000 rpm (Beckman SW50.1 rotor) to remove insoluble debris. Regardless of the type of radiolabeling employed for parasites in this WC RIP, greater than 80% of the radioactivity was recovered in the supernatant after centrifugation. Finally, 100 μl of 10% (vol/vol) protein A-bearing *S. aureus* cells prepared as previously described (2, 3, 12, 17) was added to the clarified, solubilized extract, and the mixture was again incubated at 37°C with occasional shaking. The protein A-bearing *S. aureus* cells were then pelleted and washed, and the adsorbed radiolabeled antigen was processed with dissolving buffer as described above.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The procedures outlining preparation of total *T. vaginalis* proteins for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography were as outlined elsewhere (1, 2, 17). Stacking and separating gels consisted of 3 and 7.5% acrylamide (Bio-Rad Laboratories, Richmond, Calif.), and electrophoresis was carried out with a Protean Dual 16-cm slab cell apparatus (Bio-Rad) with a constant current of 15 mA per gel. After penetration of the bromophenol blue tracking dye into the separating gel, the current was increased to 30 mA per gel. Gels were stained with Coomassie brilliant blue (Sigma), destained, and processed for fluorography as described previously (2, 17).

Agglutination of trichomonads and indirect immunofluorescence. Specific agglutination of *T. vaginalis* organisms by antibodies directed against membrane proteins was performed as recently outlined (17). Extensively washed trichomonads were incubated with a suspension of formaldehyde-fixed protein A-bearing *S. aureus* pretreated with the serum reagent also used for the RIP assay (1, 17). After incubation for various lengths of time at 37°C, samples of pellets containing agglutinated parasites and supernatants

having individual motile organisms were evaluated with dark-field optics with a Leitz Ortholux II microscope. The specificity of all sera used is as indicated in the data generated in RIP assays, and the extent of agglutination was recorded as previously described (17).

The detection of antibody to *T. vaginalis* surfaces was also attempted by using fluorescence microscopy. To correlate with results from RIP and protein A-bearing *S. aureus*-mediated agglutination, however, control normal rabbit serum and antiserum from immunized rabbits were each fractionated with protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) chromatography. A 0.1-ml sample containing approximately 2×10^6 organisms washed twice with PBS was then mixed with an equal volume of purified immunoglobulin G (IgG) fraction (protein concentration, 3 mg/ml) or serum reagents. After incubation for 20 min at 37°C with continuous gentle shaking, the parasites were washed twice with PBS and suspended in 0.1 ml of fluorescein isothiocyanate–goat anti-rabbit immunoglobulin G (Cappel Labs, Cochranville, Pa.) diluted 1:5 in PBS buffer. The suspension was placed at 4°C in the dark for 20 min before gentle washing as above. The trichomonads were finally suspended in 0.2 ml of PBS and evaluated with a Zeiss IM35 microscope equipped with fluorescence and darkfield optics. *T. vaginalis* organisms remained motile throughout this procedure.

RESULTS

Identification of immunogenic membrane proteins. Initial characterization of *T. vaginalis* membrane proteins was accomplished with a Z3-12 detergent extract of radioiodinated trichomonads as a source of antigen in the RIP assay. This was compared with a Z3-12 preparation of [^{35}S]methionine-labeled parasite proteins employed in the antibody-antigen reaction mixture. Efficient radiolabeling of most or all *T. vaginalis* proteins with [^{35}S]methionine has been accomplished and used successfully to assess the nature and extent of IgG from immunized rabbit serum for reactivity with specific immunogenic proteins (1).

Solubilized, ^{125}I-labeled trichomonal surface proteins immunoprecipitated with antiserum from immunized rabbits are shown in Fig. 1A (lane b). The absence of any radioiodinated proteins reactive with prebled control serum (lane a) demonstrates the specificity of the methodology employed. In addition, the use of antiserum extensively adsorbed with live parasites in the RIP assay resulted in decreased or absent protein bands after electrophoresis (lane c), suggesting that antibody reactive with intact, motile trichomonads was effectively removed and reinforcing the idea of the surface location for the observed protein bands (lane b). Approximately 17 proteins were detected and ranged in molecular weight (MW) from 200,000 (200K) to 20K under these experimental conditions. Alternate

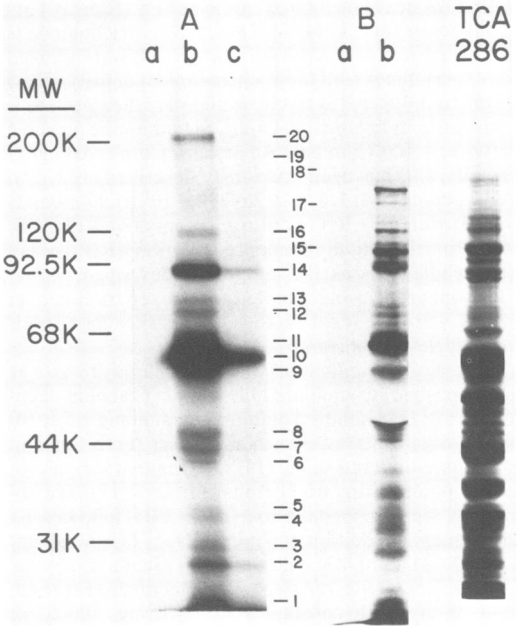

FIG. 1. Fluorogram of RIP with Z3-12–solubilized, radioiodinated (A) or ^{35}S-labeled (B) trichomonal proteins incubated with normal rabbit serum obtained before immunization (lane a), antiserum generated in rabbits immunized with *T. vaginalis* strain 286 (lane b), and antiserum adsorbed against intact, motile organisms as described in the text (lane c). TCA 286 refers to total trichloroacetic acid-precipitated trichomonal proteins used for comparative purposes.

radioiodination procedures such as lactoperoxidase-catalyzed labeling did not efficiently tag exposed proteins with Na^{125}I (data not shown), but did result in similar electrophoretic patterns after very extended exposure of X-ray film during autoradiography.

Comparative profiles of immunogenic proteins were obtained by using intrinsically labeled parasites. Incubation of ^{35}S-labeled Z3-12 286 extract with the same antiserum resulted in the immunoprecipitation of comigrating and other trichomonal proteins (Fig. 1B, lane b). In contrast, control prebled serum did not immunoprecipitate any labeled parasite proteins (lane a). Of interest was the similarity in the antiserum reactivity to both ^{125}I- and ^{35}S-labeled proteins, suggesting a parasite origin for the radioiodinated immunogenic membrane proteins of *T. vaginalis*. The pronounced intensity of protein bands with apparent MWs of 92K (protein 14) and 60K (protein 10) may be indicative of large numbers of tyrosine residues for these polypeptides. Alternately, the intensity of protein bands has been shown to correlate with the antibody titer in reactive serum (2), and intense bands obtained with radioiodinated detergent extracts

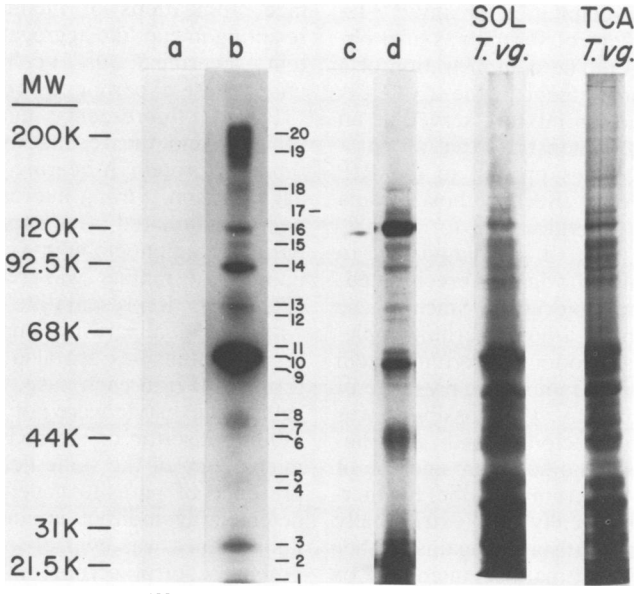

FIG. 2. WC RIP fluorograms with ^{125}I-labeled (lanes a and b) versus [^{35}S]methionine-labeled (lanes c and d) intact, motile *T. vaginalis* cells as the antigen in the reaction mixture. Organisms were incubated with sera and solubilized with Z3-12 detergent, and antibody-binding membrane proteins were immunoprecipitated with protein A-bearing *S. aureus* and processed as described in the text. Lanes a and c represent ^{125}I- and ^{35}S-labeled trichomonads, respectively, incubated with normal rabbit serum obtained before immunization. Lanes b and d represent ^{125}I- and ^{35}S-labeled parasites incubated with antiserum reagent. SOL *T. vg.* is representative of a ^{35}S-labeled Z3-12 extract used as a control to insure efficient solubilization of trichomonal proteins before the addition of protein A-bearing *S. aureus* to the reaction mixture. TCA *T. vg.* is as described for Fig. 1.

may reflect the predominance of antibody toward these membrane proteins.

A band with a MW of approximately 200K was also obtained when ^{35}S-labeled extract was used in the RIP assay, but only after prolonged exposure of the X-ray film. Because parasites radiolabeled with a ^{3}H-amino acids mixture yielded similar RIP data, these results suggest a low copy number for these high-MW proteins on trichomonal membranes. A representative control profile of detergent-solubilized, ^{35}S-labeled proteins is illustrated in Fig. 2. The protein pattern of a Z3-12 extract of ^{125}I-labeled surface proteins was not included because of the high background levels of either free Na^{125}I or other radioiodinated components such as trichomonal lipids in the acrylamide gels. Radioiodinated proteins indicative of a profile equivalent to that seen in Fig. 1A (lane b) were, however, apparent in this extract.

Antibody binding by membrane proteins with intact trichomonads. Because investigators have recently and successfully utilized whole organisms as antigen in an RIP assay (7, 8), it was equally important to determine whether, under our experimental conditions, the same or different parasite proteins would be immunoprecipitated with the IgG reagent. A successful WC RIP would greatly enhance our knowledge of highly immunogenic *T. vaginalis* surface proteins capable of binding antibody while in their native membrane orientations.

Intrinsically or extrinsically labeled trichomonads were incubated with prebled serum or antiserum from an immunized rabbit and solubilized with Z3-12 before immunoprecipitation of immune complexes with protein A-bearing *S. aureus*. Antiserum reacted with the identical reportoire of immunogenic proteins in a WC RIP with ^{125}I-labeled *T. vaginalis* (Fig. 2, lane b). Again, approximately 20 proteins with a MW spectrum of 200K to 20K were detected. Noteworthy were intense bands of apparent MW of 92K and 65K consistent with data obtained with presolubilized radiolabeled parasites as antigen (Fig. 1). In contrast to the antiserum reactivity, control prebled serum did not immunoprecipitate any radioiodinated trichomonal proteins (Fig. 2, lane a).

The almost identical protein pattern obtained with [^{35}S]methionine-labeled *T. vaginalis* in the WC RIP (lane d) confirmed the surface-exposed nature of these immunogenic proteins and demonstrated the specificity and sensitivity of this technology. The gel profile detected in this WC RIP (Fig. 2, land d) differed considerably from that obtained when presolubilized, ^{35}S-labeled organisms were employed (Fig. 1B, lane b). The

absence of immunoprecipitation of any ^{35}S-labeled proteins with control serum was also observed (Fig. 2, lane c). The demonstration of a complete protein complement in the Z3-12 extract used in the reaction mixture served as an additional control to demonstrate that all parasite proteins were always present in the RIP reaction mixture (Fig. 2). Because host plasma proteins are known to avidly bind to intact *T. vaginalis* organisms (17), it was important to establish the trichomonal origin of precipitated, radioiodinated macromolecules. Since these parasites were grown in a horse serum-supplemented medium, a radioiodinated serum protein band (Fig. 1 and 2) would imply the presence of anti-equine serum antibody. This possibility was eliminated by lack of precipitin bands after immunoelectrophoresis of horse serum and use of anti-trichomonal rabbit serum as probe. Furthermore, RIP with intrinsically and extrinsically labeled parasites and antiserum against either Trypticase-yeast extract-maltose medium or horse serum did not result in the immunoprecipitation of any radiolabeled polypeptides (unpublished observations).

Localization of surface-directed antibody. Because trichomonads possess specialized surface structures directly observable with dark-field optics, it was important to demonstrate the immunogenic potential of these readily detectable organelles. Also, because serum from immunized rabbits has been reported to agglutinate trichomonads (14, 15, 23), we felt that a highly sensitive and specific agglutination procedure previously developed in this laboratory (17) would allow us to examine whether IgG characterized by these RIP experiments was directed at these surface structures.

Protein A-bearing *S. aureus* organisms pretreated with either control serum or antiserum against strain 286 were incubated with a suspension of washed *T. vaginalis*. Thus, IgG directed against exposed membrane proteins on trichomonads might allow for protein A-bearing *S. aureus* binding and microscopic observation of immunogenic parasite structures. Figure 3 (panel 1B) clearly shows the extensive agglutination of motile trichomonads in a representative experiment when protein A-bearing *S. aureus* cells were incubated with rabbit antiserum. No agglutination or protein A-bearing *S. aureus* binding to intact organisms was detectable with protein A-bearing *S. aureus* cells pretreated with control prebled serum or protein A-bearing *S. aureus* cells alone (Fig. 3, panel 1A). Also, antibody did not appear to be directed against parasite anterior flagella (Fig. 3, panels 2A and 2B) and the posterior axostyle. Extensive clumping of protein A-bearing *S. aureus* was apparent, however, throughout the remaining trichomonal surface. Indications of membrane perturbations resulting in cap-like aggregates of adsorbed protein A-bearing *S. aureus* cells were also detected under these experimental conditions.

Finally, fluorescence microscopy was also utilized, and the results were correlated with those of protein A-bearing *S. aureus*-mediated agglutination. Strong fluorescence of trichomonads agglutinated after exposure to protein A-purified immunoglobulin G or antiserum from immunized rabbits was readily demonstrated (Fig. 4B). Representative microscopic fields with individual parasites and with different fluorescent intensities are also illustrated (Fig. 4C through 4F); in each case, the trichomonal flagella and axostyle were not apparent with either rabbit antiserum or the IgG fraction. Dark-field microscopy of the same field clearly shows the presence of parasite flagella and axostyle not detected by indirect immunofluorescence. No fluorescence was ever observed with IgG from prebled control serum or after incubation of parasites with control serum (Fig. 4, panel A), supporting previous RIP and agglutination results. Thus, antibody directed at flagella and axostyle was never detected under these experimental conditions.

DISCUSSION

T. vaginalis is a protozoan parasite responsible for trichomonal vaginitis and is responsible for one of the leading sexually transmitted diseases in this country and the world. A complex host-parasite relationship appears operative in this urogenital infection of women. For example, an intimate association between trichomonads and epithelium as well as extensive tissue cytopathology have been documented (10). Nonetheless, little or no information is available on specific virulence factors mediating disease pathogenesis. Recent efforts by this laboratory to examine the interaction between parasite and host macromolecules indicated that *T. vaginalis* possessed the ability to loosely and avidly bind plasma or tissue components (17). This information may be useful in enhancing our understanding of certain aspects of the biology of this parasite, such as trichomonal circumvention of immune surveillance mechanisms. Since an alternate strategy toward increasing our knowledge of this microorganism would be to characterize the trichomonal membrane, an attempt was made to identify highly immunogenic and exposed membrane proteins of *T. vaginalis*. This effort was a logical extension of recent work in this laboratory which examined the nature and extent of antibody directed toward immunogenic parasite proteins and produced by immunized or infected experimental animals (1).

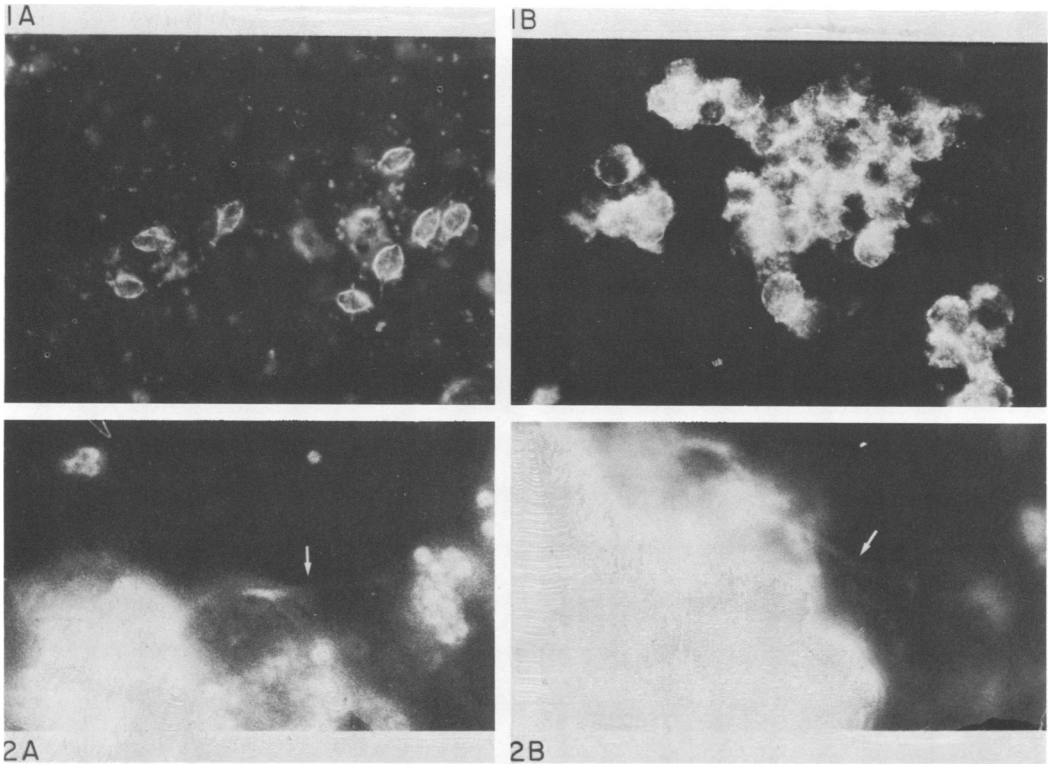

FIG. 3. Representative specific protein A-bearing *S. aureus*-mediated agglutination of live, motile trichomonads (1B) after pretreatment of staphylococci with rabbit antiserum against *T. vaginalis* strain 286. Treatment of protein A-bearing *S. aureus* with normal rabbit serum resulted in no detectable parasite agglutination (1A). Frames 2A and 2B are enlargements of individual trichomonads after agglutination emphasizing the absence of protein A-bearing *S. aureus* binding to parasite flagella (arrow). Protein A-bearing *S. aureus* pretreated with rabbit anti-286 serum was also unreactive with parasite undulating membrane and axostyle as described in the text.

Initial characterization of immunogenic trichomonal membrane proteins was accomplished by using a detergent extract consisting of *T. vaginalis* radioiodinated by chloramine-T as antigen in an RIP assay (Fig. 1). Z3-12 represents a gentle and efficient detergent system (4, 6) which allowed for specific antibody-antigen interactions. At least 17 major ^{125}I-labeled membrane proteins were found to reside on parasite membranes. Of interest was the pronounced intensity of two polypeptides with apparent MWs of 92K (protein 14) and 65K (protein 10). Previous characterization of immunogenic trichomonal proteins with intrinsically labeled detergent preparations (1) revealed a high titer of IgG directed against these two membrane proteins. Additionally, RIP performed concomitantly with these experiments, but with ^{35}S-labeled proteins, clearly affirmed the highly immunogenic nature and parasite biosynthesis of these and other trichomonal proteins (Fig. 1B, lane b). The enhanced specific activities of these two polypeptides after chloramine-T-mediated radioiodination also cannot be excluded from these initial observations. Confirmation of the surface orientation of the ^{125}I-labeled proteins immunoprecipitated with antiserum was obtained by RIP with antiserum adsorbed against live, motile organisms (Fig. 1A, lane c). The absence of protein bands in the electrophoretic profiles when control prebled serum or antiserum generated against medium components was used in the RIP assay demonstrated the high specificity of the procedures employed.

Almost identical results were obtained when whole radioiodinated trichomonads were employed as antigen in the WC RIP assay (Fig. 2) as compared with the employment of ^{125}I-labeled, presolubilized preparations. The commonality of the autoradiograms reinforced the presence of prominent antibody-binding proteins on *T. vaginalis* membranes and the highly immunogenic nature of protein 14 (92K) and protein 10 (65K). The increased intensity of a group of proteins with MWs of about 200K suggested that the prior solubilization with de-

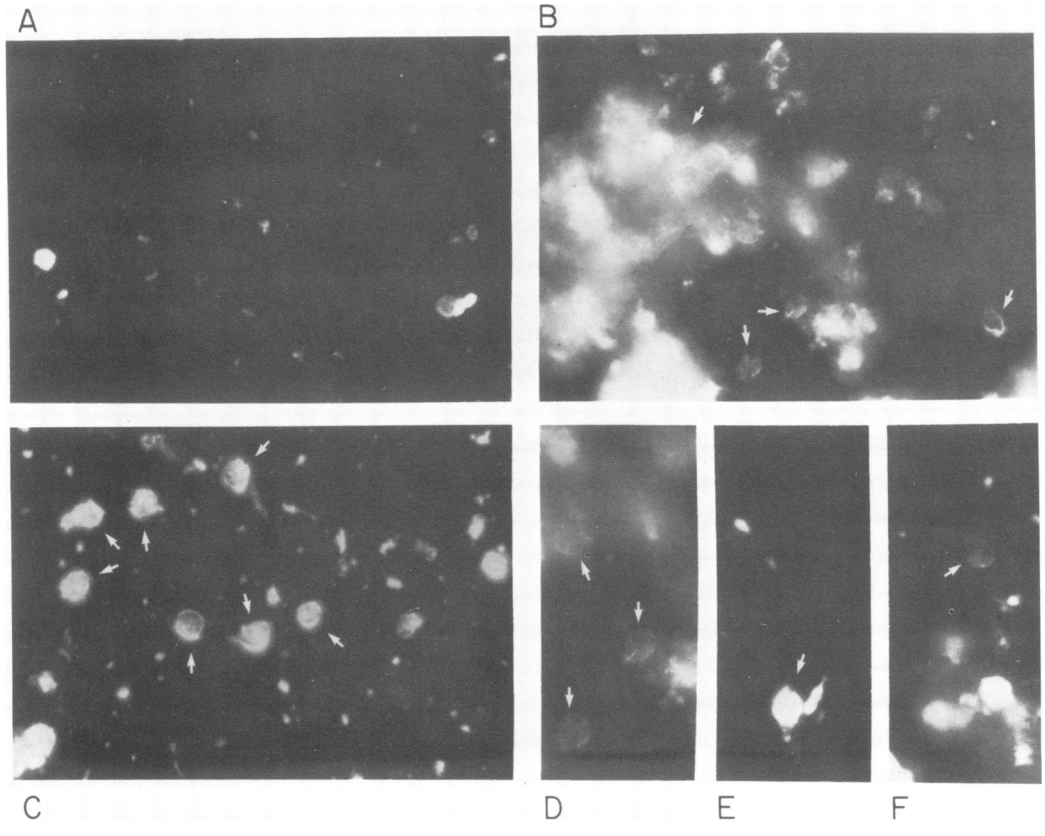

FIG. 4. Indirect immunofluorescence microscopy with intact, motile *T. vaginalis* organisms. The interaction of trichomonads with the IgG fraction from normal rabbit serum (A) and with IgG from a rabbit immunized with strain 286 (B through F) is as described in the text. The use of normal rabbit serum or serum from a rabbit immunized with *T. vaginalis* yielded similar results. Dark-field microscopy was performed on all of the same fields to insure parasite motility and presence of flagella and axostyle.

tergent systems did not allow for proper identification of immune determinants by antibody. Alterations in protein antigen quaternary structure caused by detergents might destroy antibody-reactive sites. This fact emphasizes the need for more than one methodology in assessing immunogenic parasite proteins. Especially important was the recent demonstration of proteins 10, 14, 16, and 20 as those antigens possessing highest IgG titer in antiserum from immunized rabbits or subcutaneously infected mice (1).

The identification of the same antibody-accessible proteins in the WC RIP performed with intrinsically [^{35}S]methionine-labeled organisms confirmed the parasite origin of these polypeptides (Fig. 2, lane d). Thus, antiserum immunoprecipitated ^{35}S-labeled proteins which comigrated with bands obtained when ^{125}I-labeled *T. vaginalis* organisms were used (Fig. 2, lane b). The absence of any additional proteins when intrinsically labeled trichomonads were utilized in the WC RIP indicates that the same proteins also contain sufficient tyrosine residues for subsequent RIP analysis. On the other hand, a group of proteins around 200K in MW were not readily detectable when ^{35}S-labeled parasites were employed. As with the previous experiment (Fig. 1), extended exposure of X-ray film did result in the appearance of immunoprecipitated proteins in this region. The generation of similar data with a mixture of tritiated amino acids for radiolabeling total trichomonal proteins (data not shown) suggests that these group of proteins are present in very few copies per cell. Finally, as addressed above, the proteins identified as immunogens exposed on *T. vaginalis* membranes were additionally determined to be of trichomonal origin through lack of immune complexes formed by (i) immunoelectrophoresis of horse serum using anti-*T. vaginalis* serum as a probe and (ii) RIP of radiolabeled trichomonal proteins with antiserum raised against medium components or horse serum.

Highly immunogenic, antibody-accessible membrane proteins of *T. vaginalis* may be grouped into these possessing MWs greater than 100K (proteins 16 through 20) and those possessing MWs between 65K and 100K (proteins 9 through 15). Lower-MW species were detected, but usually gave weaker bands after autoradiography (Fig. 2). Future studies detailing the molecular organization of these proteins while within the membrane will be useful in ascertaining the structure and function properties of these surface proteins and characterizing the importance of the lower-MW immunogens. As with previous studies in our laboratory (1), no differences in the makeup of antibody-accessible proteins were detected in clones (9) of *T. vaginalis*. Thus, possible heterogeneity among *T. vaginalis* populations (10, 11, 21, 22) cannot be attributed to instability of these immunogenic membrane proteins. These conclusions, however, must be interpreted with caution until trichomonads examined immediately after isolation are compared with the same cultures allowed to grow for extended periods of time. Thus, the antigenic variability of in vitro-grown parasites is presently under investigation. Of importance would be studies aimed at elucidating the biological function of these highly immunogenic trichomonal surface proteins.

Finally, the demonstration that immunoglobulin from rabbit antiserum was not directed toward specialized parasite membrane structures is extremely noteworthy (Fig. 3 and 4). Antibody reactive with either axostyle or flagella was not detected using two distinct indicator systems. These initial RIP data along with agglutination and fluorescence studies are important for dissection of IgG-targeted proteins with potential as vaccinogen candidates and is consistent with published reports on the immunology of trichomonal infection (14, 15, 23). The highly immunogenic nature of *T. vaginalis* membrane proteins which react with antibody while the proteins are in their native state makes them presumptive candidates for either experimental vaccines or immunodiagnostic reagents. This effort, however, is dependent upon future analysis of antibody responses in serum and vaginal secretions among infected women with varied symptomology.

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