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SOLUBLE *TRICHOMONAS VAGINALIS* ANTIGENS IN CELL-FREE CULTURE SUPERNATANTS

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Trichomonad proteins present in cell-free supernatants from logarithmic phase cultures of a patho
strain of *Trichomonas vaginalis* were detected with enzyme-linked immunosorbent assay employing a
dy from mice and rabbit antisera. Numerous immunogenic membrane proteins of *T. vaginalis*
identified by immunoblotting of culture filtrate with rabbit antisera. Released membrane antigens ra
in molecular weight from 30 000 to 300 000 under reducing conditions were also detected in radioimm
precipitation assays using growth medium from mid-logarithmic phase organisms which were extrins
labeled. Parallel experiments using [^{3}H]thymidine-labeled *T. vaginalis* demonstrated that lysis wa
solely responsible for the presence of parasite proteins in growth medium.

Key words: *Trichomonas vaginalis*; Immunogens; Released proteins

INTRODUCTION

Trichomoniasis, a sexually-transmitted disease of the urogenital tract, is cause
the protozoan, *Trichomonas vaginalis*. While much is known about the sheddi
membrane fragments during normal growth and multiplication by both bacteria
eukaryotic cells [1–5], no such information is available on *T. vaginalis*. The relea
membrane antigens by certain pathogenic protozoa has also been described [
Since microbial components released into the host environment can have co
quences for parasitized individuals [1,2] and because knowledge of antigens in
charge fluids of infected humans might help the development of probes for impr
diagnosis of trichomoniasis [8], we performed experiments to identify proteins p
bly released into culture medium. We present here data which indicate a relea
soluble and highly immunogenic membrane proteins by *T. vaginalis*.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IMS, infected mouse serum; IRS, in
rabbit serum; NET, NaCl-EDTA-Tris-HCl buffer; NMS, normal mouse serum; NRS, normal rabbit s
PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RIP, radioimmunopr
tation; SDS, sodium dodecyl sulfate.

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In lieu of filtration, supernatants were centrifuged at 20 000 × *g* for 15 min at 4
Either procedure for clarification of possible large fragments in supernatant yiel
similar results in ELISA.

Parasites were monitored throughout growth to ascertain continuous multipli
tion and motility of organisms. The supernatant was removed without disturbing
pellet, and incubation of medium inoculated with supernatant did not result in grow
of trichomonads, indicating no organisms in the 18 h culture supernatant.

Quantitation of parasite protein antigen released into culture supernatant was a
accomplished by the ELISA method described above. Mean absorbance reading
quadruplicate culture supernatant were compared to standard curve protein val
obtained using a soluble extract of *T. vaginalis* [11]. Solubilization of trichomon
with Zwittergent 3–12 detergent was as described [11] except that no ultracentrifu
tion of the extract was performed. Concentration of protein was determined by
Bradford assay (Bio-Rad Laboratories, Richmond, CA), and protein levels from 0
mg ml^{-1} to 8.00 mg ml^{-1} added to IgG-coated microtiter wells as above yielde
reproducible standard curve. For this part of our study, supernatant was added
IgG-coated wells as above except the incubation was for 2 h at 37°C. The wells w
washed three times followed by addition of undiluted rabbit antisera and incubat
for 1 h at 37 °C. After addition of alkaline phosphatase-conjugated goat anti-rab
IgG and incubation for 1 h at 37°C, wells with substrate were incubated for 20 m
Absorbance was recorded as described above.

Immunoblotting of parasite antigens found in supernatant. Samples were collecte
various times of growth after inoculation of medium with *T. vaginalis* and trichon
nads removed by centrifugation at 500 × *g* at 4°C for 10 min. Supernatant
removed without disturbing the pellet and filtered as before. Each time point
samples of decreasing volume, but each volume contained the same number
organisms (2 × 10^7). Filtered supernatant was then loaded onto a 2 × 10 cm Affi-
Blue-agarose (Sigma Chemical Co., St. Louis, MO) column for chromatograp
removal of albumin. The nonadherent fraction was then subjected to protein
Sepharose (Sigma) chromatography for removal of IgG. The unbound material
then dialyzed and lyophilized, and each dried sample was dissolved in 1 ml distil
water. 10 μl of this material was diluted 1/10 in PBS and combined with a two-f
concentrated electrophoresis dissolving buffer [9], boiled, and used for SDS-PA
The immunoblotting procedure was performed at least eight times using differ
preparations of supernatant for each experiment.

Electrophoretic transfer of proteins from acrylamide gels was as recently describ
[14]. Briefly, following incubation of blots with 5% BSA prepared in a 150 mM Na
mM EDTA-50 mM Tris-hydrochloride (NET), pH 7.2, buffer [11], control or anti
vaginalis serum diluted 1/5 in NET was added to the blots. Antiserum used wa
mixture of equal volumes of serum from rabbits immunized individually against str
NYH 286 and strain ATCC 30236 (JH31A) [11]. This was performed because of

PAGE for purity, and the anti-trichomonad IgG reactivity was established by RI
previously detailed [10,11]. Normal rabbit serum and pooled sera from normal
served as controls [10,11].

RESULTS

ELISA detection of T. vaginalis NYH 286 antigen in culture supernatant. An EL
using IgG antibodies generated in experimental animals was used initially for de
ting trichomonad antigens possibly present in culture supernatant. The spe
binding of parasite proteins present in logarithmic phase cultures to polyvinylchlo
wells coated with IgG from infected mouse sera is presented in Fig. 1A. Rabbit ant
diluted 1/1 000 but not 1/10 000 contained sufficient antibody for detecting anti
Immunized rabbit sera and goat anti-rabbit IgG antibody coupled to alkaline p
phatase did not react with IgG-coated wells incubated with uninoculated mediun
expected, wells coated with the same amount of IgG from normal mouse sera gav

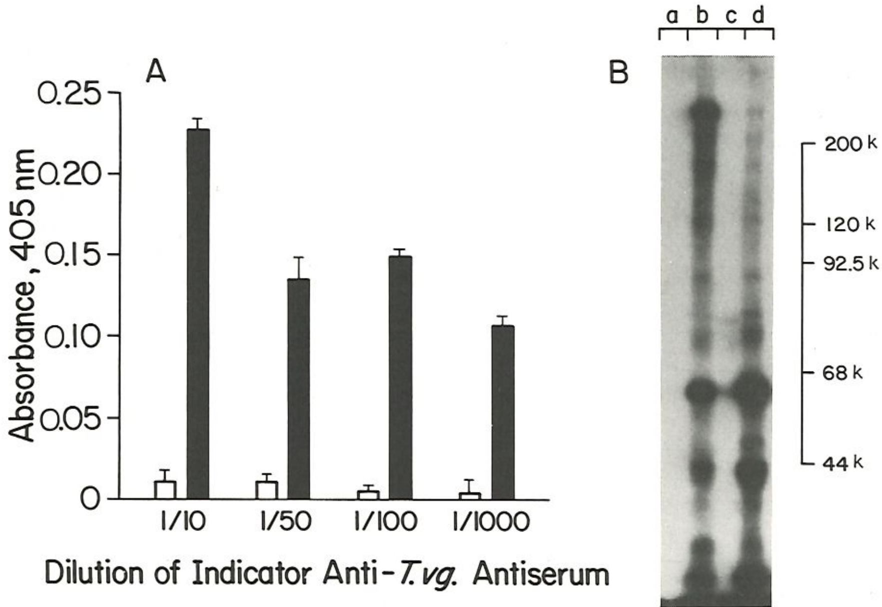

Fig. 1. Binding of *T. vaginalis* 286 proteins by ELISA (A) and soluble RIP (B). A shows the absorbar
405 nm achieved by the detection of *T. vaginalis* material in uninoculated medium (□) or culture supern
(■) by the IgG fraction of pooled infected mouse serum (IMS) fixed onto polyvinyl chloride wells foll
by reaction with immunized rabbit antiserum (IRS) and goat anti-rabbit Ig coupled to alkaline pho
tase, respectively. (B) demonstrates the reactivity of mouse (lane b) and rabbit (lane d) antisera, respect
with specific *T. vaginalis* 286 membrane proteins present in a soluble detergent extract of radioiodi
organisms. Reactivity of normal mouse and rabbit sera are shown in lanes a and c, respectively. Numb
right of gel profiles refer to the migration of molecular weight standards.

experiments in Fig. 2, protein bands were detected in supernatant of mid-logarit
phase organisms (12 h) and late-logarithmic phase organisms (24 h) [9]. Incre
reactivity and a greater number of bands was observed at 30 h. This time point w
least 6 h prior to microscopic detection of dead trichomonads or substantial relea
radioactivity from [^{3}H]thymidine-labeled organisms (Table II). No bands were
with normal rabbit serum (data not shown). The absence of bands at 0 h indicates
little or no damage was done to the parasites by the experimental procedures. A
rabbit antisera did not possess any reactivity to medium components. Consistent
RIP data (Fig. 1B), rabbit antisera recognized a set of protein bands in an immuno
of total trichloroacetic acid-precipitated proteins [9] from approximately 1.3×1
vaginalis (Fig. 2C) which served as a positive control. Finally, no bands were dete
when the antisera extensively adsorbed with live, motile organisms was used
equivalent immunoblots (data not shown), indicating a surface origin of antigen
culture supernatant.

Assuming the lysis of 2×10^7 organisms for each time point of the experim
trichomonad antigen would then be present in the supernatant. Considering a

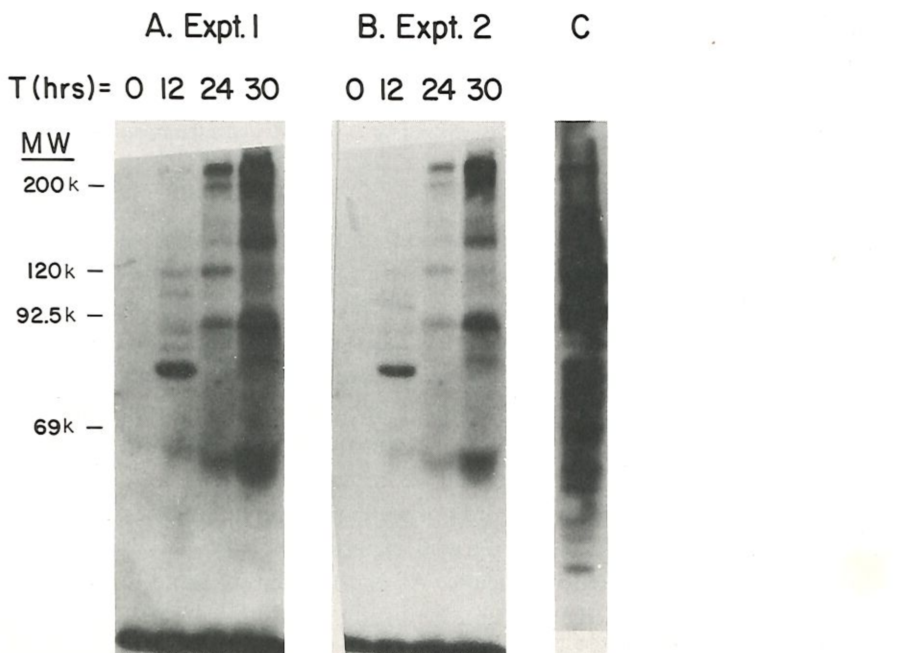

Fig. 2. Immunoblot detection of *T. vaginalis* antigens in culture supernatant in two representative
course experiments (A and B). Bands represent parasite proteins detected by pooled immunized r
antiserum to *T. vaginalis* strains 286 and JH31A (ATCC 30236). (C) demonstrates the immunodetecti
antigens using a blot of total trichloroacetic acid-precipitated proteins equivalent to 1.3×10^6 *T. vag*
after SDS-PAGE. MW refers to molecular weights of protein standards.

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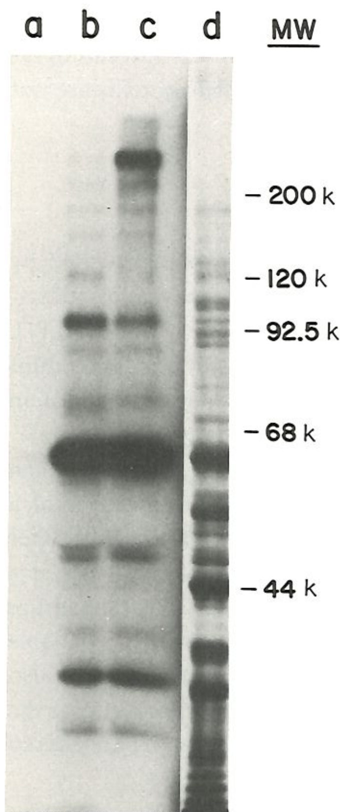

Fig. 3. SDS-PAGE-autoradiogram of RIP with radioiodinated *T. vaginalis* 286 proteins present in cul
supernatant and as described in Materials and Methods with normal rabbit serum (lane a) and antise
generated in rabbits immunized with *T. vaginalis* strain NYH 286 (lane b) and JH31A (ATCC 30236) (
c). Lane d shows a typical Coomassie brilliant blue stained protein pattern of trichloroacetic acid-precip
ed proteins. MW refers to molecular weights of protein standards.

Identification of antigens by RIP. We then employed a different procedure
identifying parasite components shed into the medium. Supernatant after growth
extrinsically-labeled organisms was processed similarly as for immunoblotting. I
munoprecipitation of trichomonad proteins was then accomplished by incubation
supernatant with sera from rabbits individually immunized with strains 286 (lane
and JH31A (lane c), respectively, followed by addition of protein A-bearing *Staphy
coccus aureus* (Fig. 3). Autoradiograms after SDS–PAGE revealed numerous radio
dinated proteins bound with antibody. Interestingly, a more intense band with
molecular weight greater than 200 000 was detected using heterologous JH31A anti
ra. Importantly, no protein bands were detected with normal rabbit serum (lane
The use of extensively adsorbed antisera failed to immunoprecipitate iodina
proteins in culture supernatant (data not shown), and these data are in agreement w

compositions and host immune responses to individual strains of *T. vagina*
[11,13,15–17] is of interest and requires further evaluation.

The data from this study do not allow for differentiation between a spontaneo
release of membrane antigens versus an energy-dependent secretory mechanis
Clearly, more research is needed to address this aspect of our findings. Nonethele
the identification of highly immunogenic membrane proteins released by *T. vagina*
is important for numerous reasons. These trichomonad antigens may elicit inflamm
tory reactions [18] or other processes of cellular immunity and thus contribute
virulence and disease pathogenesis [19–21]. Furthermore, a continuous turnover a
release of highly immunogenic parasite proteins may neutralize antibody or cytoto
lymphocytes present in the urogenital region during trichomoniasis [13,16,17], th
short-circuiting specific anti-*T. vaginalis* defense mechanisms. Equally meaningfu
the possible deposition of these highly immunogenic parasite proteins on host tissu
with resultant host cytopathology due to immune surveillance. Finally, the paras
proteins in culture supernatants may represent key antigens found in secretions
individuals with urogenital trichomoniasis. This knowledge may be useful in t
development of an immunodiagnostic test [8,22] through generation of monoclo
antibody to specific trichomonad antigens possibly present in vaginal wash or muco
reagents.

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