CONCISE COMMUNICATIONS

Identification of *Trichomonas vaginalis* α-Actinin as the Most Common Immunogen Recognized by Sera of Women Exposed to the Parasite

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A study on presence of antibodies to *Trichomonis vaginalis* in serum was done on a group of 500 pregnant, asymptomatic Angolan women. A serologic screening, done by ELISA, revealed that 41% of the women had IgG and IgM against the parasite. Analysis of sera by immunoblotting revealed that 94.4% of sera with anti-*T. vaginalis* IgG class antibodies were reactive against a common immunogenic protein of 115 kDa. The common immunogen was identified as the protozoan α-actinin. All sera recognizing the 115-kDa antigen were reactive against both native and recombinant *T. vaginalis* α-actinin and nonreactive against human α-actinin. The findings presented in this work offer a new tool for epidemiologic studies and open new perspectives for vaccination.

Trichomoniasis is the most diffused nonviral sexually transmitted disease worldwide [1], characterized by a severe discomfort with inflammation and abdominal pain [2], although the infection may often be asymptomatic, leading to underestimation of the disease diffusion. During pregnancy, trichomoniasis can lead to adverse pregnancy outcome, manifested with preterm delivery and low birth weight [3]. *Trichomonas vaginalis* induces humoral, secretory, and cellular immune responses in infected individuals [4]. Several studies, aimed at characterizing prominent immunogens [5], have assessed the high degree of antigenic heterogeneity and phenotypic variability of the parasite. The humoral response seems to be directed mainly against common determinants [4], but most of them have not yet been clearly identified and characterized. Moreover, most of the previous studies had been performed by using a low number of samples, all of which had been obtained from women with an active trichomonad infection.

To identify the common immunogens, we performed a serologic study in a highly endemic area for trichomoniasis over a wide group of pregnant women, enrolled independently because of symptoms of vaginal infection.

Materials and Methods

Cell cultures, organisms, and patients. Human fibroblasts (from human embryonic lung), recombinant *Escherichia coli* cells, and 11 *T. vaginalis* clinical isolates were cultured, as described elsewhere [6]. A group of 500 pregnant asymptomatic women aged 15–40 years attending the Lucrecia Paim Hospital in Luanda, Angola, was enrolled for this study. All women were subjected to standard clinical examination, and vaginal samples were collected, examined for presence of *T. vaginalis* by wet mount, and processed for identification of vaginal microorganisms. A 10-mL blood sample was collected from each patient. Ten sera from women with acute trichomoniasis and 10 sera from healthy male volunteers were collected in Italy.

ELISA. ELISA was done according to the method reported by Alderete [7]. Isolate SS-22, which is not infected by *Mycoplasma hominis* [8], was chosen as source of antigen to avoid nonspecific antimycoplasma reactivities. All sera were tested for presence of IgG or IgM against the parasite at a dilution of 1 : 50 in PBS plus 5% nonfat dry milk (blotto). Sera were classified as negative if the ELISA optical densities (ODs) in the wells were lower than the cutoff value, which was established as twice the mean value obtained with sera from 10 healthy male volunteers; as weakly positive if OD was 2–3 times the cutoff value; as intermediate if OD was >3–4 times the cutoff value; and as strongly positive if OD was >4 times the cutoff value.

SDS-PAGE and Western blotting. Total *T. vaginalis* SS-22 protein preparations were obtained by trichloroacetic acid precipitation [9], electrophoresed by SDS-PAGE, and transferred onto a nitrocellulose membrane, which was probed with 500 mL of each diluted serum. All Angolan sera and the 10 sera from Italian women

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affected by trichomoniasis were diluted in blotto as follows: 1 : 200 for weak, 1 : 300 for intermediate, and 1 : 400 for strongly positive sera. As a control, 20 sera from Angolan women negative for *T. vaginalis* by both ELISA and wet-mount but positive for other sexually transmitted diseases (STDs), including candidiasis, syphilis, gonorrhea, hepatitis B, and AIDS, and 10 sera from healthy Italian male volunteers were tested at a dilution of 1 : 100. After incubation, membranes were washed and incubated with anti-human IgG or IgM rabbit immunoglobulins conjugated with alkaline phosphatase (Sigma, St. Louis). Bound antibodies were detected with chromogenic substrates.

Detection of T. vaginalis *recombinant and native α-actinin with human sera.* Recombinant *T. vaginalis* α-actinin was obtained as described elsewhere [10]. Immunoprecipitation of the native *T. vaginalis* α-actinin was performed according to standard procedures [11]. Total proteins from *E. coli* expressing the recombinant α-actinin and the native, immunoprecipitated protein were electrophoresed and transferred onto nitrocellulose. The membranes were both probed with 20 sera positive for the 115-kDa *T. vaginalis* antigen and 10 ELISA-negative sera. After 2 h of incubation, membranes were incubated with secondary anti-IgG antibodies for 1 h. Bound antibodies were then detected as described earlier. Prior to use, the human sera were preadsorbed with *E. coli* total proteins.

Competition and cross-reactivity studies. Six nitrocellulose lanes containing the native immunoprecipitated *T. vaginalis* α-actinin, transferred as described, were incubated with 3 human sera positive for anti–115-kDa *T. vaginalis* antigen or with 3 negative human sera. After 2 h, the membranes were incubated with rabbit anti–*T. vaginalis* α-actinin antibodies. After another 2 h, the membranes were incubated for 1 h with anti-rabbit secondary antibodies.

The experiment was also performed by inverting the incubation order of rabbit and human antibodies. Membranes were first incubated for 2 h with rabbit anti–*T. vaginalis* α-actinin antibodies. The 3 115-kDa–positive or the 3 negative human sera were then applied and incubated for 2 h. Anti-human IgG secondary antibodies were then added and incubated for 1 h. As a control, another lane was probed only with rabbit anti–*T. vaginalis* α-actinin antibodies, a second lane only with a *T. vaginalis*–positive human serum, and a third lane only with a negative human serum. After 4 h, the membranes were incubated with the respective secondary antibodies and then treated for detection of bound antibodies.

To assess absence of cross-reactivity of the anti–*T. vaginalis* α-actinin antibodies with human α-actinin, a total of 5×10^6 trypsinized, washed human embryonic lung cells were subjected to SDS-PAGE and transferred onto a nitrocellulose filter, which was incubated for 2 h with 10 anti–*T. vaginalis*–positive sera, 10 negative sera, and commercial anti–α-actinin antibodies (Sigma). After washing, secondary antibodies were added to the wells and incubated for 1 h. The filter was then washed and developed.

Results

T. vaginalis organisms were microscopically observed in 78 of 500 vaginal samples, corresponding to 15%. Among all 500 sera, 203 (41%) tested positive by use of the ELISA test: 108 of them had IgG (53%), 61 had IgM (30%), and 36 (17%) had both. Among the 78 women who tested positive by the wet-mount test, the percentage of seropositivity rose to 86% (67 women). In this case, 43 (64%) had IgG, 10 (15%) had IgM, and 14 (21%) had both. No positive correlation was found between antibody titer or immunoglobulin class and wet-mount positivity.

The antibody specificity was investigated by immunoblotting. We observed that 94.4% of the 144 IgG-positive sera recognized a *T. vaginalis* protein weighing 115 kDa. The percentage rose to 98% in women positive by use of the wet-mount test. Another common immunogen, weighing ~140 kDa, was detected in 76.8% of all sera and 90.3% of sera from women positive by the wet-mount test. A specific reactivity against the 115-kDa polypeptide was also detected in 9 of 10 sera from Italian patients with trichomoniasis. The 115- and 140-kDa common immunogens were expressed by all 10 *T. vaginalis* isolates of different geographic origin that were tested. Reactivity against a number proteins of lower molecular weight was also observed, but it showed a high sera-to-sera variability (figure 1).

Less selective reactivities were observed by using IgM-positive sera: ~50% of sera were reactive against an antigen weighing 40 kDa. Presence of an antigen of this molecular weight recognized by IgM in sera of infected patients is consistent with results of previous studies [5]. Our group had recently cloned the *T. vaginalis* α-actinin [10]. Because this protein has the same molecular weight as the prominent 115-kDa antigen, is constantly expressed, and is present in all isolates, we investigated whether there was identity between the 2 proteins. All 115-kDa antigen-positive human sera tested recognized the recombinant α-actinin, whereas all negative controls did not. No reactivity was observed against the recombinant *E. coli* lacking the *T.*

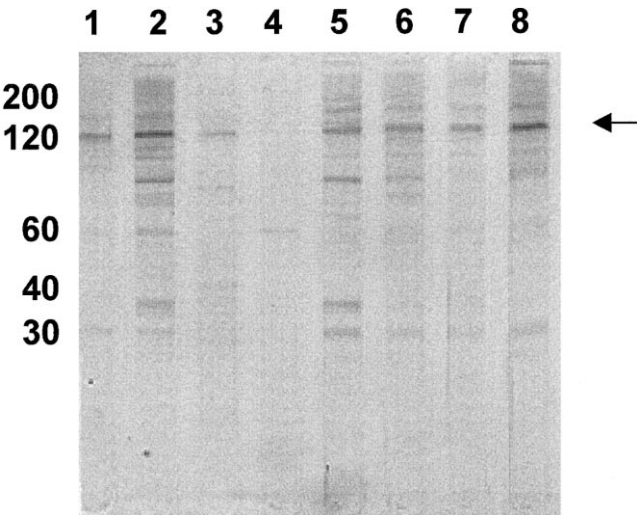

Figure 1. Immunoblot patterns of 8 representative Angolan sera with high IgG response obtained by probing a total *Trichomonas vaginalis* trichloracetic acid protein preparation. Molecular weight markers are shown on the left. The arrow points to the common 115-kDa antigen.

vaginalis α-actinin cDNA. The same results were obtained with the native *T. vaginalis* α-actinin.

To further confirm the identity between *T. vaginalis* α-actinin and the 115-kDa *T. vaginalis* antigen, immunoprecipitated native protozoan α-actinin was used for a competition experiment among 115-kDa antigen–reactive human sera and monospecific rabbit anti–*T. vaginalis* α-actinin antibodies. Preincubation of *T. vaginalis* α-actinin with the rabbit antibodies abolished binding of the positive human sera (figure 2*A,* lane 3). Moreover, when *T. vaginalis* α-actinin was preincubated with the 115-kDa–positive human sera, binding of rabbit antibodies was abolished as well (figure 2*B,* lane 3), whereas preincubation with negative human sera did not abolish binding of anti-α-actinin rabbit antibodies. This indicated a competition between human antibodies specific for the common 115-kDa antigen and rabbit anti-*T. vaginalis* α-actinin antibodies, therefore confirming the identity of the 115-kDa common antigen as the protozoan α-actinin.

We recently observed that anti–*T. vaginalis* α-actinin rabbit antibodies are not reactive with the human form of the protein or with cytoskeletal proteins from other parasites [10]. This observation led us to investigate whether anti–*T. vaginalis* α-actinin antibodies in human sera are directed against epitopes absent in human α-actinin. Therefore, human sera showing a positive reaction with both recombinant and native *T. vaginalis* α-actinin were probed with human fibroblast total proteins. The sera tested failed to recognize the human form of α-actinin, readily recognized by commercial anti–α-actinin antibodies.

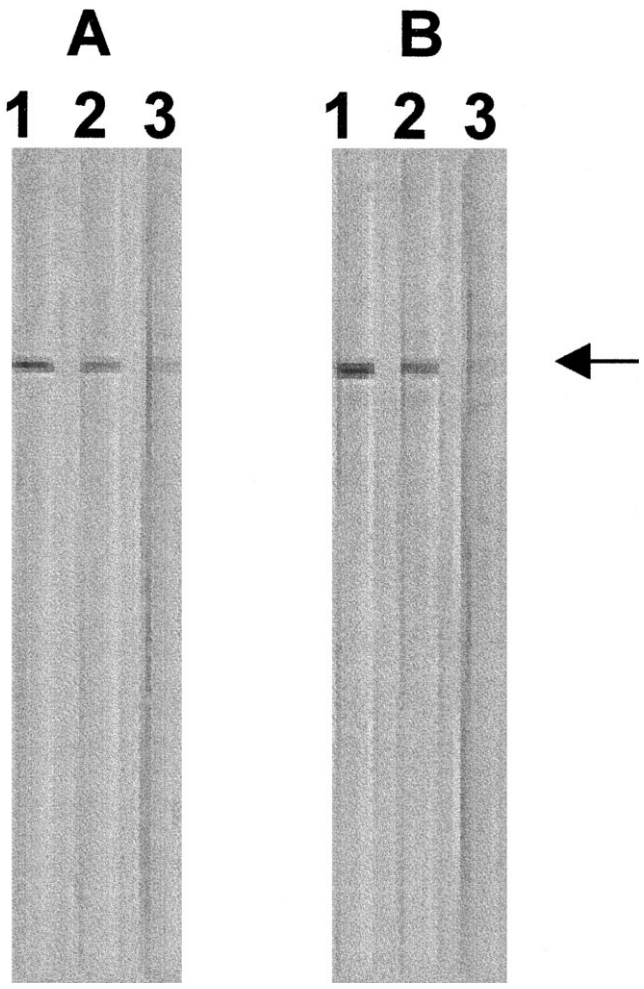

Figure 2. Representative blots showing the 2 competition experiments performed on immunoprecipitated native *Trichomonas vaginalis* α-actinin. Lanes 1 and 2 represent the immunoblot pattern obtained, respectively, with rabbit anti–*T. vaginalis* α-actinin antibodies and with a 115-kDa–positive human serum, whereas the third lanes represent the result of the competition between the 2 antibodies. *A,* lane 1: rabbit anti–*T. vaginalis* α-actinin antibodies; lane 2: positive human serum; lane 3: rabbit anti–*T. vaginalis* α-actinin antibodies followed by positive human serum and then by anti-human IgG immunoglobulins. *B,* lane 1: rabbit anti–*T. vaginalis* α-actinin antibodies; lane 2: positive human serum; lane 3: positive human serum followed by rabbit anti–*T. vaginalis* α-actinin antibodies and then by anti-rabbit immunoglobulins. For details, see Materials and Methods. The arrow points to *T. vaginalis* α-actinin.

Discussion

To identify the parasite proteins that elicit a common humoral response in people exposed to the parasite, we analyzed a group of 500 sera obtained from pregnant women living in a highly endemic area for STDs, including trichomoniasis. A screening by ELISA revealed a 41% seropositivity for IgG and IgM. No positive correlation was found between presence of IgM antibodies and wet-mount positivity; presence of anti–*T. vaginalis* immunoglobulins in serum appears to have no relationship with a current parasite infection and therefore seems not to be useful for diagnosis.

Western blotting analysis revealed that 94% of the IgG-positive sera reacted against a protozoan protein weighing 115 kDa. This percentage rose to 98% when sera of women positive by use of the wet-mount test were considered. Presence of a common antigenic molecule with this molecular weight is consistent with findings from previous reports [5, 12]. The 115-kDa antigen was identified as the protozoan α-actinin [10, 13]. Identification of α-actinin as a common immunogen has important implications, because it is a structural protein, is constantly expressed during parasite life, is present in all isolates, and does not undergo geographic variation.

As reported elsewhere [10–14], α-actinin is constantly observed on the surface of epithelial cells lysed by the parasite and thus is exposed to immunologic recognition; this might explain the high antibody response observed against this intracellular protein. It seems intriguing to observe that the common immunogen seen on 70% of sera has a molecular weight of 140 kDa, corresponding to that of another *T. vaginalis* protein observed to bind to target cells [14].

In rabbits immunized with total parasite proteins, α-actinin

elicits an important humoral immune response [10]. The competition assay performed in this work revealed that rabbit and human anti–*T. vaginalis* α-actinin antibodies are directed against the same epitopes on the protein. These are unique to the protozoan α-actinin; in fact, anti–*T. vaginalis* antibodies present in human sera do not react with the human form of the protein.

The immunogenicity of *T. vaginalis* α-actinin and the lack of serologic reactivity against the human form are consistent with the molecular structure [10, 13]: in fact, the homology with other α-actinins is not found in the central region of the molecule, which also contains 3 high immunogenic regions [10]. Further studies could be performed to show whether multiple isoforms of this gene exist; in fact, some *T. vaginalis* proteins are encoded by slightly divergent genes [2].

No humoral response against the parasite protein is detectable in sera obtained from healthy donors or from women affected by vaginal infections other than trichomoniasis; presence of anti–*T. vaginalis* α-actinin IgGs in serum can therefore be considered a good epidemiologic marker. Further studies on this common immunogen, such as the subcloning of the highly immunogenic peptides located on the central region of the protein, might represent a starting point for designing a new vaccine strategy for prevention of trichomoniasis [15] or for serologic studies on diffusion of this widespread STD.

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