**Trichomonas vaginalis** Infection Induces Vaginal CD4⁺ T-Cell Infiltration in a Mouse Model: A Vaccine Strategy to Reduce Vaginal Infection and HIV Transmission

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**Background.** Complications related to the diagnosis and treatment of *Trichomonas vaginalis* infection, as well as the association between *T. vaginalis* infection and increased transmission of and susceptibility to human immunodeficiency virus, highlight the need for alternative interventions. We tested a human-safe, aluminum hydroxide-adjuvanted whole-cell *T. vaginalis* vaccine for efficacy in a BALB/c mouse model of vaginal infection.

**Methods.** A whole-cell *T. vaginalis* vaccine was administered subcutaneously to BALB/c mice, using a prime-boost vaccination schedule. CD4⁺ T-cell infiltration in the murine vaginal tissue and local and systemic levels of immunoglobulins were measured at time points up to 4 weeks following infection.

**Results.** Vaccination reduced the incidence and increased the clearance of *T. vaginalis* infection and induced both systemic and local humoral immune responses. CD4⁺ T cells were detected in vaginal tissues following intra-vaginal infection with *T. vaginalis* but were not seen in uninfected mice. The presence of CD4⁺ T cells following *T. vaginalis* infection can potentially increase susceptibility to and transmission of human immunodeficiency virus.

**Conclusions.** The vaccine induces local and systemic immune responses and confers significantly greater protection against vaginal infection than seen in unvaccinated mice (*P* < .005). These data support the potential for a human vaccine against *T. vaginalis* infection that could also influence the incidence of human immunodeficiency virus infection.

**Keywords.** *Trichomonas vaginalis*; vaccine; HIV transmission; CD4.

*Trichomonas vaginalis* is the most common nonviral sexually transmitted pathogen [1]. Recent estimates from 2008 reported an incidence of 276 million and prevalence of 187 million *T. vaginalis* infections, increases of 11.5% and 22.2%, respectively, from estimates in 2005 [1, 2]. Since the majority of *T. vaginalis* infections are asymptomatic in men and women, these statistics likely underestimate the actual global burden of disease [1, 3]. Typical symptoms for men include pain and inflammation of the urogenital tract. More important sequelae are infertility, benign prostatic hyperplasia, and prostate cancer [3–8]. Because of the large burden of asymptomatic disease, particularly in males, the pathogenic potential of *T. vaginalis* is underrecognized. Infections in females include symptoms such as vaginal discharge, increased vaginal pH, inflammation of the reproductive tract, and itching, and untreated infection leads to chronic infection. Sequelae of greater concern include infertility, cervical cancer, pelvic inflammatory disease, and adverse events with implications for fetal and newborn health, including preterm labor, premature rupture of membranes, and low birth weight [4, 9–14]. *Trichomonas vaginalis* infection is also correlated with increased susceptibility to and transmission of human immunodeficiency virus (HIV) [4].
The causal relationship between HIV susceptibility and transmission and \textit{T. vaginalis} infection is unclear. Modes of HIV acquisition due to \textit{T. vaginalis} infection have been speculated to be due to damage to the vaginal epithelial layer caused by inflammation, increased monocytes cell susceptibility to HIV, and bacterial vaginosis or changes in vaginal flora colonization [15–18]. Epidemiological studies report increased susceptibility to HIV when an individual is already infected with \textit{T. vaginalis} (odds ratio, 1.52–2.74) [19–21]. Increased transmission of HIV may be due to a higher level of viral shedding into the genital tract or to genital ulcers [22, 23]. Finally, higher rates of \textit{T. vaginalis} and HIV coinfection were found in pregnant women, compared with nonpregnant women (\(P < .05\)), which can increase the risk of vertical transmission of HIV [24]. A model used to estimate coinfections in the United States used an odds ratio of 1.8 and attributed 2\% of all HIV infections to \textit{T. vaginalis} infections [17]. However, this value likely underestimates the attribution of \textit{T. vaginalis} infections, owing to the prevalence of asymptomatic diseases and underdiagnosis.

Previously, we developed a mouse vaginal infection model of \textit{T. vaginalis} and showed the potential of vaccination to prevent disease and reduce the duration of infection [25]. This model used Freund adjuvant. Although \textit{T. vaginalis} infection is curable in most cases with imidazole agents, resistance to imidazole has increased, and \textit{T. vaginalis} is often unrecognized and infection undiagnosed, especially in low-resource countries. Thus, preventing infection by delivery of an affordable vaccine could have a major impact on maternal-child health and reduce HIV transmission. To address this need, we studied a \textit{T. vaginalis} vaccine that contains a human-appropriate adjuvant, aluminum hydroxide (alum), in our mouse vaginal infection model. We assessed the effect of vaccination, relative to that of no vaccination, on the initial number and duration of \textit{T. vaginalis} infections, the levels of \textit{T. vaginalis}–specific immunoglobulin G (IgG) present in serum and the local vaginal environment, and the presence of CD4\(^+\) and CD8\(^+\) T cells in vaginal tissue. Immunological data were recorded before and after infection and with and without vaccination, to assess changes in the immunological response over time.

**METHODS**

**Vaccination**

Mice were vaccinated 8 weeks (day \(−56\)) and 4 weeks (day \(−28\)) before vaginal infection (day \(0\)). The vaccine consisted of \(1 \times 10^9\) whole-cell \textit{T. vaginalis} in Freund complete/incomplete adjuvant (hereafter, “Freund”), 0.50 mg of alum (hereafter, “alum 0.50 mg”), or 0.75 mg of alum (hereafter, “alum 0.75g”). A sham vaccination group was included that received 0.75 mg of alum without whole-cell \textit{T. vaginalis} (hereafter, “alum sham”). Unvaccinated mice acted as controls. Inoculum regimen and key procedure dates followed previously established protocols [25] in our laboratory (Supplementary Table 1).

**Animals and Animal Care**

Female BALB/c mice weighing 17–19 g were obtained from Charles River (Charles River Laboratories, Montreal, Canada), housed in the animal care facilities at the University of Ottawa Roger Guindon Campus (Ottawa, Canada) in groups of 4, and randomly allocated to receive vaccine, sham vaccine, or no vaccine. All procedures performed on mice were approved by the Animal Care Committee of the University of Ottawa, Faculty of Medicine, for ethical consideration, (protocol number BMI-108).

**Blood Collection**

Blood specimens were collected from the saphenous vein 3 weeks after the prime vaccination (day \(−33\)), 3 weeks after the boost vaccination (day \(−7\)), and 2 and 4 weeks following intra-vaginal challenge with \textit{T. vaginalis}. Blood samples were stored at room temperature for 2 hours and then centrifuged for 20 minutes at 2000 g. Serum was pipetted from the collection tubes into microtubes and stored at \(-80^\circ\text{C}\).

**Vaginal Washes**

Infection status was determined on days 7, 14, and 28 after vaginal infection, by culture of vaginal washes. Culture tubes were incubated at 37\(^\circ\text{C}\) in 5\% \text{CO}_2\) and assessed for growth after 4 days.

We assessed presence of \textit{T. vaginalis}–specific total immunoglobulin G (IgG) from vaginal washes, which were performed on days 1, 3, 7, 14, 21, and 28 following intravaginal challenge with \textit{T. vaginalis}. In brief, 50 µL of phosphate-buffered saline (PBS) was pipetted gently and repeatedly up to 10 times in the vagina of mice. The liquid was aspirated and transferred to a microfuge tube containing 1 µL of a protease inhibitor cocktail (P8340, Sigma-Aldrich, St. Louis, Missouri). An additional wash was repeated for a final volume of 100 µL. This volume was centrifuged at 12 000 g for 10 minutes at 4\(^\circ\text{C}\). The supernatant was extracted and replaced with an additional 100 µL of fresh PBS with 1 µL of protease inhibitor cocktail and then rotated in an incubator at 20 rpm (orbit diameter 1.27 cm) for 2 hours at 4\(^\circ\text{C}\). Extraction was repeated for a final 300-µL volume and stored at \(-20^\circ\text{C}\).

**Enzyme-Linked Immunosorbent Assays (ELISAs)**

A laboratory preparation of \textit{T. vaginalis} (OC15) lysate was coated overnight at 4\(^\circ\text{C}\) on 96-well, high-binding cell culture plates (Costar 3595; Corning, New York) at a concentration of 2 µg/mL OC15 lysate in a total volume of 50 µL. The wash step consisted of the following: wells were emptied, washed 3 times with PBS-T wash buffer (PBS plus 0.005\% Tween 20), and dried. One hundred microliters of blocking buffer (PBS-T plus 1\% bovine serum albumin [BSA]) were added to each well. Following incubation of blocking buffer, plates were washed. For serum samples, the wells were then filled with 100 µL of PBS-T with 0.1\% BSA and 0.5 µL of undiluted serum. For vaginal wash samples, 40 µL of PBS-T with 0.1\% BSA and 60 µL of vaginal wash extract were added to each
well. The plates were incubated for 2 hours at room temperature and then washed. Peroxidase-conjugated goat anti-mouse monoclonal antibodies (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) were added in a total volume of 100 µL of PBS-T to each well at the following dilutions: 1:10 000 IgG, 1:5000 IgG2a, and 1:7000 IgG1. The plates were incubated for 2 hours at room temperature and then washed. Excess suppressor was decanted, and tissues were incubated for 10 minutes at room temperature with 1% BSA in TBS as a suppressor. Excess suppressor was decanted, and diluted rat anti-mouse CD8+ cells (1:100; anti-rat IgG; catalog no. 559286; BD Pharmingen, Mississauga, Canada) or CD8+ cells (1:100; anti-mouse CD8a [catalog no. 550281]; BD Pharmingen, Mississauga, Canada) was added to the slides for 30 minutes at 4°C by submerging the slides in TBS. Next, biotinylated goat anti-rat IgG (1:200 in TBS; catalog no. 559286; BD Pharmingen, Mississauga, Canada) was added to the slides for 30 minutes at room temperature in a humidification chamber. Wash steps were repeated. To quench endogenous peroxidases, the slides were blocked with 6% H2O2 in methanol for 10 minutes. A single wash step was performed. An ABC complex kit (catalog no. 32052; Thermo Scientific, Rockford, Illinois) was incubated on tissues for 45 minutes. Two washes were performed. The peroxidase activity of the ABC kit was resolved using a betazoid DAB chromogen kit (BioCare Medical, Concord, California) placed on the tissues for 5 minutes, which were then washed with distilled water. Finally, slides were counterstained, dehydrated, cleared, and mounted.

**Immunohistochemical Analysis**

Vaginas were harvested from mice euthanized on days −1, 1, 3, 7, 14, 21, and 28. Tissues were embedded in Tissue-Tek OCT (Sakura Finetek, Torrance, California) by flash freezing. Serial duplicate vaginal tissues cut 7-µm thick across the lumen (Sakura Finetek, Torrance, California) were thawed onto Superfrost Plus Gold microscope slides (catalog no. 22-035813; Fisher Scientific, Ottawa, Canada) and stored at −80°C.

Tissues were dried and fixed with acetone. Next, the tissues were rehydrated in Tris-buffered saline (TBS; pH 7.6) for 10 minutes. TBS was decanted, and tissues were incubated for 10–20 minutes at room temperature with 1% BSA in TBS as a suppressor. Excess suppressor was decanted, and dilute rat anti-mouse primary antibodies in TBS were added for detection of either CD4+ T cells (1:100; anti-mouse CD4 [catalog no. 550280]; BD Pharmingen, Mississauga, Canada), or CD8+ cells (1:100; anti-mouse CD8a [catalog no. 550281]; BD Pharmingen, Mississauga, Canada). Primary antibodies were incubated overnight at 4°C in a humidification chamber. Tissues were washed twice for 5 minutes by submerging the slides in TBS. Next, biotinylated goat anti-rat IgG (1:200 in TBS; catalog no. 559286; BD Pharmingen, Mississauga, Canada) was added to the slides for 30 minutes at room temperature in a humidification chamber. Wash steps were repeated. To quench endogenous peroxidases, the slides were blocked with 6% H2O2 in methanol for 10 minutes. A single wash step was performed. An ABC complex kit (catalog no. 32052; Thermo Scientific, Rockford, Illinois) was incubated on tissues for 45 minutes. Two washes were performed. The peroxidase activity of the ABC kit was resolved using a betazoid DAB chromogen kit (BioCare Medical, Concord, California) placed on the tissues for 5 minutes, which were then washed with distilled water. Finally, slides were counterstained, dehydrated, cleared, and mounted.

**Statistical Analyses**

Data were analyzed by the Fisher exact test and 1-way analysis of variance with the post-hoc Tukey multiple comparisons test (GraphPad Prism v5.04; GraphPad Software). P values of <.05 were considered statistically significant. A cutoff for detection of vaginal immunoglobulin was set as 2 times the standard deviation of the absorbance readings from unvaccinated mice, where values above the cutoff were considered positive readings. A z test for proportions was used to test for significant differences in the proportion of CD4+ T cells detected in tissues between days 1–14 and 21–28 (α = 0.05).

**RESULTS**

**Effect of Vaccination on Vaginal Infection**

Unvaccinated mice had significantly more infections on day 7, compared with mice in the Freund and alum groups (P < .005). Pooled results are from 5 separate experiments (Table 1). Vaccine efficacy, calculated on the basis of infections on day 7, was 64%, 61%, and 72% for the Freund, alum 0.50, and alum 0.75 groups, respectively. The number of *T. vaginalis* infections in the unvaccinated group on day 28 was also significantly greater than that in the vaccinated groups (P < .005). A total of 100%, 63%, 75%, and 23% of infections detected on day 7 were cleared by day 28 in the Freund, alum 0.50, alum 0.75, and unvaccinated groups, respectively.

**Table 1. Trichomonas vaginalis Detection by Culture Following Vaginal Wash**

<table>
<thead>
<tr>
<th>Organism Cultured, Day of Culture</th>
<th>Mice With Recovered Organisms, Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freund</td>
</tr>
<tr>
<td><em>L. acidophilus</em> wash, day −1</td>
<td>7/14 (50)</td>
</tr>
<tr>
<td><em>T. vaginalis</em> wash 1, day 7</td>
<td>4/14 (29)*</td>
</tr>
<tr>
<td><em>T. vaginalis</em> wash 2, day 14</td>
<td>2/14 (14)*</td>
</tr>
<tr>
<td><em>T. vaginalis</em> wash 3, day 28</td>
<td>0/14*</td>
</tr>
</tbody>
</table>

Groups of mice received subcutaneous prime and boost vaccinations of Freund complete/incomplete adjuvant (Freund), 0.50 mg of aluminum hydroxide (alum 0.50), or 0.75 mg of aluminum hydroxide (alum 0.75) with 1 × 10⁶ trichomonads; were unvaccinated; or received alum 0.75 without trichomonads (alum sham).

* P < .005, by the Fisher exact test, compared with the unvaccinated group.
Vaccinated mice had fewer initial infections on day 7 after vaginal infection and greater clearance of initial infections by day 28 after vaginal infection, compared with unvaccinated mice. By day 28, the percentages of mice with vaginal infection were 0% in the Freund group, 12% in the alum 0.50 group, 6% in the alum 0.75 group, 20% in the alum sham group, and 61% in the unvaccinated group.

Serum and Vaginal Immunoglobulin Levels
Mice immunized with adjuvant and trichomonads had significantly higher serum levels of total IgG and IgG1 (P < .05) at all time points, compared with mice in the unvaccinated and alum sham groups (Figure 1). High serum levels of total IgG and IgG1 were maintained 28 days following intravaginal T. vaginalis challenge in the Freund, alum 0.50, and alum 0.75 groups. Total IgG and IgG1 detection was nearly absent at each time point for the unvaccinated and alum sham groups. Serum IgG2a responses were not detected in the unvaccinated or alum sham groups but could be detected in some mice from the Freund, alum 0.50, and alum 0.75 groups (Supplementary Figure 1). The IgG2a response in the Freund group was significantly greater (P < .05) than that in the alum 0.75, alum sham, and unvaccinated groups 3 weeks after receipt of the boost vaccination. The IgG2a response in the Freund group was significantly greater (P < .05) than that in the alum sham and unvaccinated groups 4 weeks after infection. The cumulative effect of subcutaneous immunization of mice with adjuvant and T. vaginalis antigen was a pronounced total IgG and IgG1 response with variable IgG2a production. Vaginal IgG was detected almost exclusively in vaccinated mice (Figure 2 and Supplementary Figure 2).

Immunohistochemical Detection of CD4+ and CD8+ T Cells in Vaginal Tissues
Immunohistochemical staining was performed on vaginal tissue to investigate cell-mediated immune response in the vaginal tissues (Table 2 and Figure 3). Distribution of cells in
the vaginal tissue was not uniform. Therefore, viewing only 1 field of view at 40× original magnification would not provide an accurate assessment of the presence of cells in the tissue. A control, unvaccinated, and uninfected mouse that received neither lactobacillus inoculation nor T. vaginalis challenge had no CD4+ or CD8+ T cells identified in its vaginal tissue.

CD4+ T cells were not detected in any of the 5 uninfected, lactobacillus-inoculated tissues, regardless of vaccination status. CD4+ T cells were, however, detected in mice after vaginal infection. In the first 2 weeks after vaginal infection, CD4+ T cells were detected in sampled tissues from 66% (6 of 9), 64% (7 of 11), 75% (6 of 8), 63% (5 of 8), and 33% (3 of 9) of mice in the Freund, alum 0.50, alum 0.75, sham, and unvaccinated groups, respectively. However, 3–4 weeks after vaginal infection, CD4+ T cells were detected in sampled tissues from 50% (2 of 4), 0% (0 of 3), 20% (1 of 5), 25% (1 of 4), and 29% (2 of 7) of mice in the Freund, alum 0.50, alum 0.75, sham, and unvaccinated groups, respectively. No CD8 labeling beyond background was detected in any of the tissues sampled (Figure 3).

The CD4+ T-cell response was associated with vaginal challenge with T. vaginalis, and vaccinated mice had CD4+ T cells detected more often in the first 2 weeks following infection. This response, however, did not predict the presence of a positive T. vaginalis culture. The CD4+ T-cell prevalence proportions between days 1–14 and days 21–28 were significantly different for the alum 0.50, alum 0.75, and sham groups (P < .05).

**DISCUSSION**

Trichomonas vaginalis infection in humans and experimental animal models does not result in protection against reinfection [25, 26]. Indeed, reinfection is often seen in those at high risk of reexposure to T. vaginalis, including sex workers, people who engage in risky sexual behaviors, and women whose sex partner has not been treated for T. vaginalis infection [27–30]. Additional infections can be attributed to a lack of adherence to treatment, as well as to treatment failure [31, 32]. Repeat and untreated T. vaginalis infections are particularly problematic in

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Figure 2. Vaginal immunoglobulin G response on days 7 (A), 14 (B), 21 (C), and 28 (D) in one experiment, as measured by enzyme-linked immunosorbent assay (ELISA). Each data point represents a single mouse. Groups of mice received subcutaneous prime and boost vaccinations of Freund complete/incomplete adjuvant (Freund), 0.50 mg of aluminum hydroxide (alum 0.50), or 0.75 mg of aluminum hydroxide [alum 0.75] with 1 × 10^6 trichomonads; were unvaccinated; or received alum 0.75 without trichomonads (alum sham). A cutoff was set as 2 standard deviations above the mean calculated from unvaccinated mice. Values above this cutoff are considered positive.
pregnant women because of the risk of adverse pregnancy outcomes and the increased susceptibility to infection with and transmission of HIV [4, 12, 19, 33].

A vaccine would be particularly ideal for preventing the asymptomatic carriers, who are reservoirs of *T. vaginalis* infection. Vaccination against the bovine trichomoniasis, *Tritrichomonas foetus*, has been successful in preventing infections in young bulls and reducing the duration of infection in heifers. The vaccine results in greater pregnancy rates and successful births, and it produces preputial, vaginal, uterine, and systemic IgG1, IgG2, and immunoglobulin A anti-trichomonad antibody responses [34–38].

This study used the same animal model and vaccination procedures as Abraham et al [25], except for our use of an adjuvant formulation safe for human vaccination. Since the use of Freund adjuvant is not safe for human applications, we aimed to evaluate a vaccine formulation with an adjuvant that is approved as safe for humans. Using the OC15 *T. vaginalis* isolate, derived from a symptomatic patient, we produced a simple whole-cell vaccine with two different concentrations of aluminum from a premade aluminum hydroxide adjuvant (Alhydrogel). The alum vaccines (alum 0.50 and alum 0.75) and the Freund vaccine had significantly fewer initial infections measured on day 7 after intravaginal challenge with *T. vaginalis*, compared with unvaccinated mice (*P* < .005).

Alum contact with dendritic cells (DCs) stimulates an increase of ICAM-1 and LFA-1, which are CD4+ T-cell adhesion molecules, and has an antigen-nonspecific function [39]. If either the primed DCs or alum crystals can migrate to vaginal tissues, then the DCs in vaginal tissue could have tighter associations to present *T. vaginalis* antigen for the first time, compared with unvaccinated mice. This could explain the unexpected low numbers of initial infections and clearance of infection in the alum sham group, compared with the unvaccinated group. Nevertheless, vaccinated mice were significantly protected from infection on day 7 after infection, compared with nonvaccinated mice, and were more likely to clear infection by day 28.

The presence of anti-trichomonal immunoglobulin during infection has been documented in humans [40]. The role of immunoglobulin remains unclear because virulence factors of *T. vaginalis* are capable of degrading immunoglobulin [41] and may prevent complement activation or antibody-dependent cell-mediated cytotoxicity [42]. To our knowledge, epitope specificity of antibodies in humans has not been investigated and could be another potential reason for ineffective natural and amnestic immune responses in humans. Although immunoglobulin is detected during an active vaginal infection in humans, its presence does not seem to afford protection during or after infection. Perhaps immunoglobulin levels during natural infection are ineffective, but immunoglobulin levels achieved after vaccination may be protective, suggesting that the mode of antigen presentation has a differential effect on protection.

Overall, the local and systemic immunoglobulin results we report coincide with the report by Abraham et al [25] that immunoglobulin responses are detected at higher levels in serum after vaccination, compared with findings for controls, and that levels are particularly high after vaginal infection. Also, vaginal IgG is more likely to be detected in vaccinated mice, compared with controls, following vaginal infection. Alum vaccination was as effective at producing serum and vaginal immunoglobulin responses as Freund vaccination. This shows that the viability of a human-adapted *T. vaginalis* vaccine is a possibility for future protection studies.

The detection of a local humoral immune response could suggest locally produced antibodies or antibodies arising from systemic circulation. Other innate or adaptive immune mediators may be at play to influence clearance of infection, since not all mice that cleared infection had vaginal immunoglobulin levels above the cutoff.

Paintlia et al [43] isolated lymphocytes from vaginal cervical tissue obtained from mice challenged with a symptomatic isolate, those challenged with an asymptomatic isolate, or those not challenged (uninfected control). The process to isolate lymphocytes uses enzymes and dissociation medium to release lymphocytes from the excised tissues. However, peripheral blood lymphocyte contamination is a concern owing to the presence of small blood vessels, which cannot be excised prior to

### Table 2. Detection of CD4+ T Cells in Murine Vaginal Tissue Sections

<table>
<thead>
<tr>
<th>Tissue Harvest Time</th>
<th>Freund Alum 0.50</th>
<th>Alum 0.75</th>
<th>Alum Sham</th>
<th>Unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before infection</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Day 1</td>
<td>2/2</td>
<td>2/3</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Day 3</td>
<td>2/2</td>
<td>0/2</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Day 7</td>
<td>0/3</td>
<td>3/3</td>
<td>2/2</td>
<td>1/2</td>
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<tr>
<td>Day 14</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
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<tr>
<td>Day 21</td>
<td>1/2</td>
<td>0/1</td>
<td>1/3</td>
<td>1/4</td>
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<tr>
<td>Day 28</td>
<td>1/2</td>
<td>0/2</td>
<td>0/2</td>
<td>1/3</td>
</tr>
</tbody>
</table>

Groups of mice received subcutaneous prime and boost vaccinations of Freund complete/ incomplete adjuvant (Freund), 0.50 mg of aluminum hydroxide (alum 0.50), or 0.75 mg of aluminum hydroxide (alum 0.75) with *T. vaginalis*; were unvaccinated; or received alum 0.75 without trichomonads (alum sham). Samples were obtained before infection (day −2) and at various time points after *Trichomonas vaginalis* challenge (days 1, 3, 7, 14, 21, and 28). Tissues were screened in duplicate from serial sections. Tissues from vaccinated groups contained CD4+ T cells more frequently than tissues from the unvaccinated group from days 1 to 14 after infection. Additionally, CD4+ T cells were less frequently detected over time in vaccinated groups, whereas unvaccinated mice had a consistent frequency of CD4+ T-cell detection.

In addition to the lactobacillus-inoculated mice euthanized, an untreated mouse without lactobacillus inoculation or challenge with *T. vaginalis* was euthanized for immunohistochemical analysis and consequently categorized as having no prevalent CD4+ T cells.
treatment. The proportion of lymphocytes could be skewed. Their study reported the percentage of the total population of lymphocytes in vaginal cervical tissue that were CD4+ T cells. Mice with asymptomatic isolate infections had a significantly greater proportion of CD4+ T cells than uninfected mice and mice infected with symptomatic isolates \((P < .001)\). No differences existed between percentages of CD8+ T cells between isolates and controls.

Using an immunohistochemical approach, Reighard et al [44] found significantly higher counts of CD4+ T cells \((P < .05)\), B cells \((P < .05)\), and plasma cells \((P < .01)\) in human endometrial tissue, compared with uninfected controls. No differences were observed with respect to CD8+ T cells. The endometrium is not the primary site of infection. \(T. vaginalis\) is predominantly a vaginal infection, and conclusions about lymphocytes in the vaginal environment cannot be inferred from findings of endometrial analysis.

To overcome these methodological issues, we performed immunohistochemical analyses on a mouse vaginal infection model. This histological approach gives a more definite idea of where and how many lymphocytes are located within the tissue. Also, immunohistochemical assessment of the impact of vaccination on the presence of lymphocytes following a \(T. vaginalis\) infection has not been reported. We used an immunohistochemical staining procedure to investigate frozen vaginal samples obtained at various time points after infection from vaccinated and unvaccinated mice. There was a lack of CD4+ T cells in specimens obtained before infection, regardless of vaccine status. After infection, we found CD4+ T cells in tissue specimens from at least half of the mice in the vaccinated and the alum sham groups but in specimens from 33% of mice in the unvaccinated group. With the small number of samples tested at various time points, we could not determine the kinetics of the CD4+ T-cell presence after infection or after clearance of infection because the vaginal infection is not uniformly distributed, and it is possible to miss areas of T-cell infiltration or presence. However, it is clear that \(T. vaginalis\) induces a CD4+ T-cell presence in vaginal tissue. This effect has potential implications on the transmission and acquisition of HIV.

CD4+ T cells were more likely to be detected in vaccinated mice and in greater numbers within the vaginal tissue. Mice in the alum and alum sham groups had a reduction in CD4+ T cells after 2 weeks after infection. The alum vaccine efficacy was 61%–72%. Furthermore, 63%–75% of infections on day 7 were cleared by day 28 among mice in the alum groups, compared with unvaccinated mice, among which only 23% cleared...
infections by day 28. Unvaccinated mice with chronic *T. vaginalis* infection face a potentially more hazardous situation than vaccinated mice in terms of HIV transmission and susceptibility, owing to the long-term presence of CD4⁺ T cells in the vaginal tissues, compared with the brief period of influx of CD4⁺ T cells after vaccination. Thus, reducing *T. vaginalis* infection will decrease the vaginal CD4⁺ T-cell count and likely reduce the risk of HIV acquisition, despite the transient increase in the number of CD4⁺ T cells associated with vaccination. However, the transient CD4⁺ T-cell count increase must be considered with caution. Exposure of a vaccinated individual to a sex partner who is coinfected with HIV and *T. vaginalis* could result in an increased risk of HIV infection for the vaccinated individual. Consistently, CD8⁺ T cells do not appear to play a role in *T. vaginalis* infection or immune response, at least in this murine model.

An alum-adjuvanted *T. vaginalis* vaccine resulted in vaginal protection and induced clearance of infection and a reduction in the vaginal CD4⁺ T-cell count 2 weeks after infection. Thus, the attraction of an affordable *T. vaginalis* vaccine that can impact maternal-child health by reducing *T. vaginalis* and that may reduce HIV transmission and acquisition merits serious future consideration.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

*Acknowledgments.* J. D. S. designed the study, conducted data collection and data analysis, and wrote the manuscript. G. E. G. supervised the project, edited the manuscript, and approved the final manuscript.

*Potential conflict of interest.* Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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