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Short Report: Differences in Complement-mediated Killing of *Entamoeba histolytica* Between Men and Women—An Explanation for the Increased Susceptibility of Men to Invasive Amebiasis?

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Abstract. Men are more than 7 times more likely to develop amebic liver abscess or amebic dysentery caused by *Entamoeba histolytica* than women. Because the complement system could play a key role in controlling amebiasis, we determined whether serum from men and women differ in the ability to kill amebic trophozoites. We found that serum from women was significantly more effective in killing *E. histolytica* trophozoites than serum from men, and this killing was complement dependent. Our results provide a possible explanation for the differential susceptibility of men and women to amebic liver abscess and amebic colitis.

Amebic dysentery and amebic liver abscess caused by the protozoan parasite *Entamoeba histolytica* are important causes of morbidity and mortality worldwide.[1] Interestingly, the prevalence of amebic liver abscess and amebic dysentery is between 5- and 7-fold higher in men than women.[2] The reason for the greater susceptibility to disease in men is unknown, particularly because there does not seem to be a difference between men and women in the rates of intestinal colonization by *E. histolytica*.[2] To invade through the intestinal mucosa and survive within the portal circulation to establish infection within the liver, *E. histolytica* trophozoites must resist killing by complement.[1] We tested the hypothesis that differences between men and women in the efficiency of complement-mediated killing of amebic trophozoites might explain the varying susceptibility of the sexes to amebic liver abscess and amebic colitis.

We obtained serum samples from 100 consecutively enrolled healthy volunteers from an area that is not endemic for amebiasis, and examined the efficiency of their sera in killing amebic trophozoites. Among the volunteers were 66 women and 34 men, who provided 84 samples (16 were technically unsuitable for study) for analysis. Informed consent was obtained from all individuals, and this study was approved by the Washington University Institutional Review Board and Human Research Protection Office. Serum samples were screened for anti-amebic antibodies using immunoblotting;[3] no samples in this population were positive.

Complement-mediated killing of axenically cultured amebic trophozoites was measured by FACS assay using a modified version of the method of Hamelmann and others.[4] In brief, the serum samples were centrifuged at 5000 × g, and the supernatant aliquoted and stored at −70°C. Heated-inactivated serum (56°C for 30 min) was prepared as a control. *E. histolytica* HM1:IMSS trophozoites were harvested at the logarithmic phase of growth were chilled on ice for 10 minutes, pelleted by centrifugation at 500 × g for 5 minutes, washed twice with cold phosphate-buffered saline (PBS), and resuspended in PBS at cell concentration of 2×10^6/mL; 35 μL of the amoeba were mixed with 50 μL NHS and PBS to a final volume of 100 μL. The tropohozoites at a final cell concentration of approximately 5×10^5/mL were incubated for 30 minutes at 37°C, then 20 μL 0.2M EDTA was added to stop the reaction. After centrifugation at 950 × g for 2 minutes, the trophozoites were resuspended in 200 μL PBS including 0.01M EDTA and 0.5 μg/mL propidium iodide (PI, Sigma, Saint Louis, MO). The samples were incubated in the dark at room temperature for 5 minutes, and lysis was determined using flow cytometry (FACSCalibur, BD Biosences, Franklin Lake, NJ). Five thousand events were counted, and the data was analyzed with FACSscan Research Software. As previously reported, the viable trophozoites show a high green self-fluorescence monitored in channel (FL1, 530 nm) (see gated populations in Figure 1A) whereas killed or damaged trophozoites are seen as a population with high fluorescence in channel 3 (FL3, 650 nm) indicating PI staining, and as populations that have lost both autofluorescence and PI uptake.[4] Specific lysis was calculated as previously described.[4]

We found that serum samples from women were significantly more efficient in killing *E. histolytica* trophozoites than serum samples from men (Figure 1 and Table 1). Heat-inactivated serum (lacking complement activity) was significantly less efficient in killing trophozoites than untreated serum, but did have greater killing than buffer alone (Table 1), suggesting there is a small component of complement-independent killing of trophozoites by serum. Mean trophozoite killing was not different for heat-inactivated serum from men and women, indicating that the complement-independent component of serum killing does not differ between the sexes (Table 1). Killing occurred via complement, as the addition of EDTA that blocks total complement activity (Table 1), significantly inhibited killing by both female and male serum samples. The mean activity of serum from men and women did not differ in either alternative pathway or classic pathway mediated hemolysis of sensitized sheep red blood cells (data not shown). Although our sample size was relatively small (51 Caucasians, 25 African Americans, and 8 Asians) we did not see an independent effect of race/ethnicity on the efficiency of serum in killing trophozoites, nor was age (50 and above compared with under 50) a factor among men, women, or the entire population (data not shown).

Although there is overlap in activity between the populations, our study indicates that sera from females are more effective in killing amebic trophozoites via complement than sera from males. Whether this difference contributes to the

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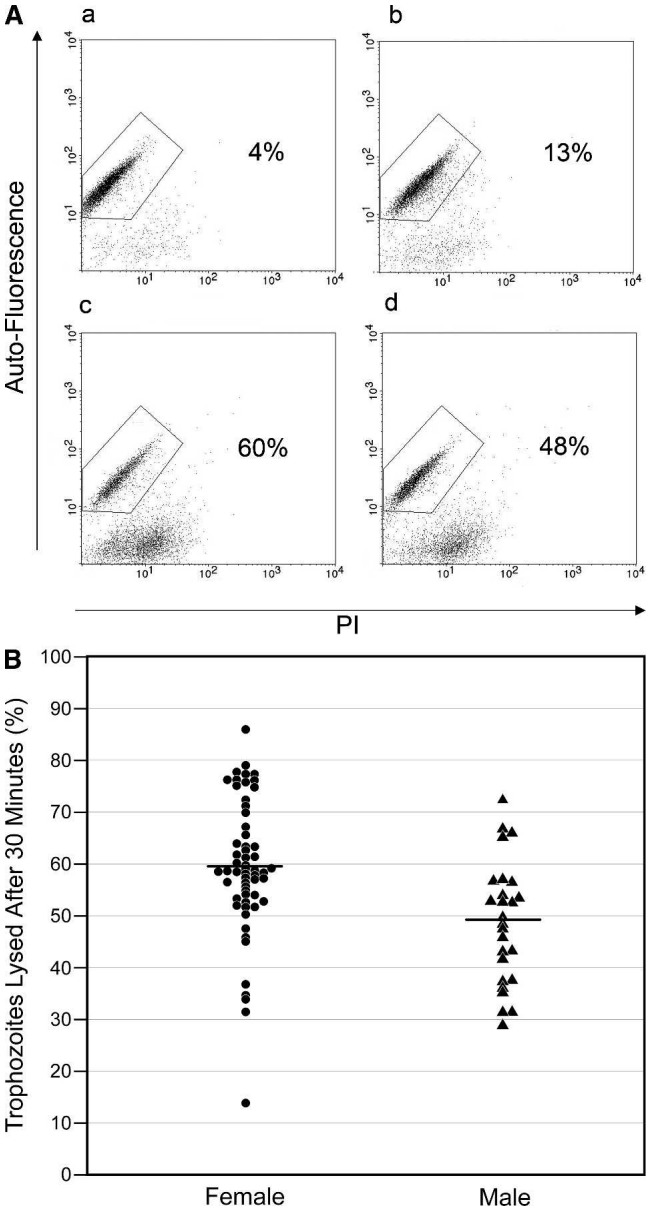

FIGURE 1. Serum from men and women differ in their ability to kill *E. histolytica* trophozoites. **A**, Amebic trophozoites of strain *E. histolytica* HM-1:IMSS were incubated with serum samples for 30 minutes at 37°C and analyzed by FACS with killed trophozoites identified by loss of endogenous autofluorescence and acquisition of propidium iodide (PI) staining.[4] Examples of assays measuring killing of trophozoites incubated with: buffer alone showing 4% killing (trophozoites outside the gated area) (panel a); heat-inactivated serum showing 13% killing (panel b); serum from a female subject showing 60% killing (panel c); and serum from a male subject showing 48% killing (panel d). **B**, Comparison of all female ($N = 56$) and male ($N = 28$) serum samples in killing *E. histolytica* trophozoites. The mean values for trophozoite killing as indicated by the bars were significantly different between men and women ($P < 0.001$).

increased risk for amebic liver abscess and amebic colitis in men is unknown, but it does provide a testable hypothesis for clinical studies. The mechanism underlying this difference remains undefined. Prior studies using tumor cells or red blood cells as targets suggested that pregnant or menstruating women, or women with some forms of cancer, have higher levels of serum cytolytic activity due to increased activity of the alternative pathway of complement.[5,6] None of the women in our study were known to have any of these conditions, and our finding that hemolytic activity via either the alternative or classic pathway was not different between men and women makes it unlikely that the differences we saw in *E. histolytica* killing are associated with differences in nonspecific cytolytic activity. Further studies to characterize the complement components from these subjects, particularly those sera at the extremes of *E. histolytica* killing activity, and to further examine the specificity of this interaction, may help define this mechanism and clarify its role in disease.

TABLE 1

Serum from men and women differ in the ability to lyse *E. histolytica* trophozoites

Source	% Trophozoites (mean ± SD) killed by			
	Serum	Heat-inactivated serum	EDTA-treated serum	Buffer alone ($N = 10$)
Male ($N = 26$)	48 ± 12	12 ± 9*	13 ± 7*	NA
Female ($N = 58$)	59 ± 12*	11 ± 4*	10 ± 3*	NA
Total ($N = 84$)	56 ± 14	12 ± 7*	11 ± 5*	4 ± 0.5†

$P < 0.001$ for the difference between males and females in killing by untreated serum; * $P < 0.001$ for the difference with untreated serum; † $P < 0.01$ for the difference with all serum samples.

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