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OBJECTIVES: HIV-neutralizing immunoglobulin A (IgA) and HIV-specific cellular immunity have been described in highly exposed, persistently seronegative (HEPS) individuals, but well controlled studies have not been performed. We performed a prospective, nested case-control study to examine the association of genital IgA and systemic cellular immune responses with subsequent HIV acquisition in high-risk Kenyan female sex workers (FSWs). **DESIGN AND METHODS:** A randomized trial of monthly antibiotic prophylaxis to prevent sexually transmitted disease/HIV infection was performed from 1998 to 2002 in HIV-uninfected Kenyan FSWs. After the completion of trial, FSWs who had acquired HIV (cases) were matched 1: 4 with persistently uninfected controls based on study arm, duration of HIV-seronegative follow-up, and time of cohort enrolment. Blinded investigators assayed the ability at enrolment of genital IgA to neutralize primary HIV isolates as well as systemic HIV-specific cellular IFN γ -modified enzyme-linked immunospot and proliferative responses. **RESULTS:** The study cohort comprised 113 FSWs: 24 cases who acquired HIV and 89 matched controls. Genital HIV-neutralizing IgA was associated with reduced HIV acquisition ($P = 0.003$), as was HIV-specific proliferation ($P = 0.002$), and these associations were additive. HIV-specific IFN γ production did not differ between case and control groups. In multivariable analysis, HIV-neutralizing IgA and HIV-specific proliferation each remained independently associated with lack of HIV acquisition. Genital herpes (HSV2) was associated with increased HIV risk and with reduced detection of HIV-neutralizing **CONCLUSION:** Genital HIV-neutralizing IgA and systemic HIV-specific proliferative responses, assayed by blinded investigators, were prospectively associated with HIV nonacquisition. The induction of these immune responses may be an important goal for HIV vaccines.