

Breast-feeding by infants exposed to human immunodeficiency virus type 1 (HIV-1) provides an opportunity to assess the role played by repeated HIV-1 exposure in eliciting HIV-1-specific immunity and in defining whether immune responses correlate with protection from infection. Breast-feeding infants born to HIV-1-seropositive women were assessed for HLA-selected HIV-1 peptide-specific cytotoxic T lymphocyte interferon (IFN)-gamma responses by means of enzyme-linked immunospot (ELISpot) assays at 1, 3, 6, 9, and 12 months of age. Responses were deemed to be positive when they reached  $\geq 50$  HIV-1-specific sfu/ $1 \times 10^6$  peripheral blood mononuclear cells (PBMCs) and were at least twice those of negative controls. A total of 807 ELISpot assays were performed for 217 infants who remained uninfected with HIV-1 at approximately 12 months of age; 101 infants (47%) had at least 1 positive ELISpot result (median, 78-170 sfu/ $1 \times 10^6$  PBMCs). The prevalence and magnitude of responses increased with age ( $P = .01$  and  $P = .007$ , respectively); the median  $\log_{10}$  value for HIV-1-specific IFN-gamma responses increased by 1.0 sfu/ $1 \times 10^6$  PBMCs/month ( $P < .001$ ) between 1 and 12 months of age. Of 141 HIV-1-uninfected infants with 1-month ELISpot results, 10 (7%) acquired HIV-1 infection (0/16 with positive vs. 10/125 [8%] with negative ELISpot results;  $P = .6$ ). Higher values for  $\log_{10}$  HIV-1-specific spot-forming units at 1 month of age were associated with a decreased risk of HIV-1 infection, adjusted for maternal HIV-1 RNA level (adjusted hazard ratio, 0.09 [95% confidence interval, 0.01-0.72]). Breast-feeding HIV-1-exposed uninfected infants frequently had HIV-1-specific IFN-gamma responses. Greater early HIV-1-specific IFN-gamma responses were associated with decreased HIV-1 acquisition.