Infection with human T lymphotropic virus type II (HTLV-II) has been linked to an increased incidence of bacterial pneumonia. To determine whether HTLV-II infection is associated with impaired humoral immune responses, we immunized a cohort of HTLV-II–infected subjects and matched uninfected control subjects with 23-valent pneumococcal polysaccharide and tetanus toxoid vaccines. The pneumococcal polysaccharide vaccine elicited comparable and significant increases in concentrations of IgG against all 5 serotypes tested at 1 and 6 months after immunization in both groups. The avidity and opsonophagocytic functions of the anticapsular IgG were similar. The concentrations of tetanus toxoid–specific IgG also increased comparably and significantly over time in both groups. Thus, HTLV-II–infected persons develop robust humoral responses to potentially protective polysaccharide and protein vaccines.

Human T lymphotropic virus type II (HTLV-II) is a member of a family of mammalian retroviruses that have similar biological properties and tropism for T lymphocytes [1]. In North America, the seroprevalence of the virus ranges from 0.4% to 24% among various injection-drug-using populations [2] and approaches 0.03% among blood donors [3], with an estimated 140,000 infected persons in the United States [1]. The cellular tropism of HTLV-II was initially reported to be toward CD8+ T lymphocytes; however, subsequent studies have demonstrated that CD4+ T lymphocytes, B lymphocytes, and monocytes can carry the proviral genome in infected persons [4, 5]. Thus, HTLV-II–related immune defects may extend to multiple components of the immune system, each of which may contribute to diminished protection against infectious diseases if functionally impaired. Indeed, both prospective and retrospective analyses have revealed that HTLV-II infection is associated with an increased incidence of bacterial pneumonia [6, 7].

Defense against Streptococcus pneumoniae, the most common cause of community-acquired bacterial pneumonia—particularly in persons with immune dysfunction—requires an intact humoral immune system that can generate an antibody response to the pneumococcal polysaccharide capsule [8]. Adults with normal pulmonary clearance mechanisms are at much lower risk for pneumonia, because macrophages and neutrophils readily phagocytose and kill the organism in the presence of pneumococcal capsular antibody and complement. In contrast, persons with altered pulmonary clearance mechanisms or with conditions associated with reduced IgG responses are at significantly greater risk for contracting pneumococcal pneumonia [9].

In the present study, we determined whether the reported predisposition of HTLV-II–infected persons to bacterial pneumonia was associated with a defective humoral immune response to specific vaccine antigens. To this end, we examined the qualitative, quantitative, and functional antibody responses of HTLV-II–infected and –uninfected persons to pneumococcal polysaccharide and tetanus toxoid vaccines.

Subjects, materials, and methods. We recruited persons enrolled in the HTLV Outcomes Study (HOST) [10] and immunized 29 HTLV-II–infected subjects (mean age, 51 years [range, 41–73 years]; 26 women and 3 men) and 21 uninfected control subjects (mean age, 50 years [range, 39–64 years]; 19 women and 2 men) with the 23-valent pneumococcal polysaccharide vaccine (Pnu-Immune 23; Lederle). The uninfected control subjects were matched to the HTLV-II–infected subjects on the basis of age, sex, and race. Of those who received the pneumococcal polysaccharide vaccine, 22 HTLV-II–infected subjects and 12 matched uninfected control subjects were also immunized with the tetanus-diphtheria toxoid vaccine (Pasteur Mérix-Coulaudt). Exclusion criteria included HIV infection, prior receipt...
of pneumococcal vaccine, splenectomy, liver disease, alcoholism, steroid use, and cancer. Blood was drawn before immunization and at 1 and 6 months after immunization, and serum samples were stored at −70°C until testing. Written, informed consent was obtained from all subjects, and protocols and experimentation guidelines were approved by the University of California at San Francisco Committee on Human Research.

Antigen-specific IgG ELISAs were performed as described elsewhere [11]. Briefly, microtiter wells were coated overnight with either 10 μg/mL pneumococcal polysaccharides type 4, 6B, 14, 19F, or 23F (American Type Culture Collection) or 2 μg/mL tetanus toxoid (Department of Health, Boston, MA), after which 2-fold serial dilutions of serum samples from paired HTLV-II–infected subjects and uninfected control subjects were added in duplicate. Each plate included either the international reference serum 89SF (provided by Carl Frasch, Center for Biologics Evaluation and Research, Food and Drug Administration) for pneumococcal polysaccharide–specific IgG or purified human IgG (Cappel-ICN) as a standard for tetanus toxoid–specific IgG. Serum for determination of concentrations of pneumococcal polysaccharide IgG was preincubated with 10 μg/mL pneumococcal cell-wall polysaccharide (Statens Serum Institut), to adsorb antibodies to cell-wall polysaccharide. After 2 h at room temperature, the presence of antibody was detected by use of horseradish peroxidase–conjugated goat anti–human IgG (Sigma). Concentrations of pneumococcal polysaccharide–specific IgG were calculated by interpolation from a standard curve derived from reference serum 89SF [12], and concentrations of tetanus toxoid–specific IgG were calculated by interpolation from a standard curve derived from the purified human IgG.

The avidity of the pneumococcal polysaccharide–specific IgG was determined in duplicate wells as described elsewhere [11]. Antibodies bound to pneumococcal polysaccharide were exposed to ammonium thiocyanate (SCN) at concentrations that ranged from 0.1 to 2.0 mol/L for 1 h. The molarity of SCN that gave 50% inhibition of optical density was calculated as the affinity index.

Opsonophagocytic killing assays were performed as described elsewhere [11]. Briefly, 2-fold serial dilutions of heat-inactivated serum were incubated with 1000 cfu of S. pneumoniae serotype 14 (ATCC 6314) for 30 min. Baby rabbit complement (10%; Cederlane Laboratories) and HL-60 phagocytic cells (American Type Culture Collection; differentiation with 120 mmol/L dimethylformamide) were added (effector:target cell ratio, 400:1) for 60 min at 37°C, after which samples were diluted in water and plated on blood agar plates. The opsonophagocytic titer was considered to be the highest dilution of serum that supported >50% killing activity, as determined on the basis of the number of viable organisms in control tubes that contained fetal calf serum, complement, and HL-60 effector cells. Serum samples with <50% killing activity at the lowest dilution tested (titer of <2) were arbitrarily assigned a titer of 1.

Statistical analyses of the differences in IgG concentrations and functional antibody responses over time and between the HTLV-II–infected subjects and uninfected control subjects were assessed by use of SAS Proc Mixed (version 9.1; SAS Institute) [13]. Because the data showed a skewed distribution, analyses were conducted on log-transformed data. For ease of interpretability, we present back-transformed least squares means that are based on the analyses of the log-transformed data. These values correspond to geometric means that are based on the raw data. We used random factors in SAS Proc Mixed to account for the matched sets of subjects and the repeated measures over time in subjects. Residual plots were used to assess assumptions of normality, linearity, and lack of outliers.

Results. Before immunization with the pneumococcal vaccine, the HTLV-II–infected subjects had significantly higher geometric mean concentrations of IgG against vaccine serotypes 4 (P = .03), 19F (P < .0001), and 23F (P = .01) than did the uninfected control subjects, whereas concentrations of IgG against serotypes 6B and 14 were similar (P > .10) (figure 1). At 1 and 6 months after immunization, both the HTLV-II–infected subjects and the uninfected control subjects generated significantly increased IgG responses to the 5 vaccine serotypes tested (P < .0001, vs. baseline values). The increases in concentrations of pneumococcal polysaccharide–specific IgG at both time points were similarly robust in both groups, as were mean increases in antibody concentrations. Thus, both the magnitude and duration of IgG responses to pneumococcal polysaccharides were substantial and comparable in healthy adults and those with concomitant HTLV-II infection.

Baseline concentrations of tetanus toxoid–specific IgG were similar in the HTLV-II–infected subjects and the uninfected control subjects (P > .10) (figure 1). Consistent with the observed robust humoral responses to polysaccharide antigens, at 1 month after immunization, both groups responded with equivalent increases in concentrations of tetanus toxoid–specific IgG, and the concentrations were significantly greater than the baseline concentrations (P < .0001). At 6 months after immunization, the concentrations of tetanus toxoid–specific IgG remained similar in both groups and were significantly higher than the baseline concentrations (P < .0001), despite a slight but statistically insignificant decrease in concentrations of tetanus toxoid–specific IgG from 1 to 6 months after immunization.

We next determined the avidity of the pneumococcal polysaccharide–specific IgG, which reflects the quality of the antigen–antibody interaction. HTLV-II infection did not compromise the avidity of the antibody produced in response to the pneumococcal vaccine. Mean baseline avidities of pneumococcal polysaccharide–specific IgG were comparable in the HTLV-II–infected subjects and the uninfected control subjects for 4 of 5
Figure 1. Least squares mean concentrations of IgG against 5 common pneumococcal capsular polysaccharide serotypes and against tetanus toxoid before immunization and at 1 and 6 months after immunization in human T lymphotropic virus type II (HTLV-II)–infected subjects and uninfected control subjects. Before immunization, the HTLV-II–infected subjects had significantly higher concentrations of IgG against pneumococcal capsular serotypes 4 (P < .03), 19F (P < .0001), and 23F (P < .01) than did the uninfected control subjects but had similar concentrations of IgG against serotypes 6B and 14 (P > .10, for both serotypes). In both groups, pneumococcal polysaccharide vaccine elicited significant increases in concentrations of IgG against all 5 serotypes (P < .0001) at 1 and 6 months after immunization, and no differences between the mean increases in antibody concentrations in each group were observed (P > .10, for all serotypes). The baseline concentrations of tetanus toxoid–specific IgG were similar in the 2 groups (P > .10). In both groups, the tetanus toxoid vaccine elicited significant and comparable increases in tetanus toxoid–specific IgG at 1 and 6 months after immunization (P < .0001, for both time points).
pneumococcal polysaccharides ($P<.0001$) (figure 2). For serotype 14, the pneumococcal polysaccharide–specific IgG from the HTLV-II–infected subjects showed a higher avidity than did that from the uninfected control subjects ($P = .02$). At 1 month after immunization, the avidities of IgG against serotypes 6B, 14, and 23F were appreciably decreased ($P < .05$), whereas the avidities of IgG against serotypes 4 and 19F were unchanged and were similar to the avidities at 6 months after

---

Figure 2. Least squares mean avidities of IgG against 5 common pneumococcal capsular polysaccharide serotypes before immunization and at 1 and 6 months after immunization in human T lymphotropic virus type II (HTLV-II)–infected subjects and uninfected control subjects. The avidity indices of the IgG against the 5 polysaccharides produced in response to the vaccine were comparable at 1 and 6 months after immunization in the 2 groups ($P > .10$, for all serotypes).
immunization. When compared at 1 and 6 months after immunization, the avidities of the specific IgG responses to the 5 pneumococcal polysaccharides were similar in both groups \((P > .10)\).

The ability of serum from the HTLV-II–infected subjects and the uninfected control subjects to support the killing of serotype 14—the serotype that is the predominant cause of invasive disease \([8]\)—in the presence of antibody and phagocytes increased significantly after immunization (figure 3). These increases in killing activity in the 2 groups were comparable and were sustained at 6 months after immunization.

**Discussion.** We have shown that persons with HTLV-II infection are competent with respect to the generation of robust and functional humoral immune responses to both polysaccharide and protein vaccines. These data are relevant because persons with this retroviral infection have increased incidences of infectious diseases \([7]\). Prominent among these diseases is bacterial pneumonia, and *S. pneumoniae* is the most common cause of bacterial pneumonia and bacteremic pneumonia in adults \([8]\). Antibodies against pneumococcal polysaccharides are an important mechanism of defense against the organism. In the present study, the HTLV-II–infected subjects produced significant increases in concentrations of IgG against each of 5 prominent pneumococcal capsular antigens after immunization. In addition to the magnitude of the responses, the quality and functional activity of these antibodies were also comparable to those of the healthy, uninfected control subjects. Indeed, the avidity of capsule-specific antibodies, as well as the ability of serum to support killing by phagocytes and complement, were comparable in the 2 groups before and after immunization. Moreover, the function and elevated concentrations of antibodies were conserved for at least 6 months. Thus, the IgG responses in the HTLV-II–infected subjects to both the pneumococcal polysaccharide and tetanus toxoid vaccines were similar to those in the uninfected control subjects.

The present study was undertaken to assess the humoral immune status of HTLV-II–infected persons in the context of the multiple reports that HTLV-II–infected persons have a higher incidence of pneumonia than do uninfected persons \([7]\). We had anticipated that immune function might be impaired by HTLV-II infection, because monocytes and both T and B lymphocytes are susceptible to HTLV-II infection. Given that the antibody responses to pneumococcal polysaccharide and tetanus toxoid vaccines in our HTLV-II–infected subjects were comparable to those in our uninfected control subjects, the results of the present study do not provide a biological basis within the humoral immune system for the reported link between HTLV-II infection and pneumonia. Other explanations may underlie the presumed discrepancy between the reported increased incidences of disease, including of pneumonia, in the literature and the integrity of the humoral responses to vaccination observed here. First, HTLV-II may perturb the host defense against pneumonia by infecting alveolar macrophages and compromising the uptake and killing of bacteria by phagocytes. Second, the existence of abnormal levels of expression of various cytokines during HTLV-II infection may contribute to impaired innate and specific defenses against pneumonia \([14]\). Third, only a subset of patients with HTLV-II infection may exhibit relevant perturbations in immune function, and it is among this subset that immune defects and related infections may be most prominent. Finally, other behavioral and biological factors—such as cigarette smoking, injection drug use, age, crowding, and other comorbidities—should be considered to be independent risk factors for disease in patients with HTLV-II infection \([7]\).

In the present study, before immunization with the pneumococcal vaccine, the HTLV-II–infected subjects had a higher concentration of IgG against 3 of 5 pneumococcal polysaccharides than did the uninfected control subjects. These data suggest that the HTLV-II–infected subjects may have had a higher rate of prior exposure to pneumococcal strains expressing those 3 polysaccharides than did the uninfected control subjects or that the duration of the IgG responses to these capsular polysaccharides was sustained more consistently in the HTLV-II–infected subjects. In addition, the HTLV-II–infected subjects may have had higher levels of nonspecific antibodies that are reactive toward pneumococcal polysaccharides—as has been
reported for HIV-infected adults [15]—and this may also have contributed to the higher baseline concentrations.

Our analysis of the functional activity of pneumococcal polysaccharide–specific IgG was based on both opsonization and avidity assays. Although the opsonophagocytic titer of serum directed against a type 14 pneumococcal strain increased significantly and comparably in both groups, a corresponding decrease in IgG avidity for type 14 was observed in both groups. The lack of an increase in the avidity of type 14 pneumococcal polysaccharide–specific IgG, which may have been due to the relatively high baseline avidity exhibited by both groups at the time of immunization, is consistent with most previous findings in adults [11]. Nonetheless, on the basis of the results of the opsonophagocytic killing assay, the present study provides evidence that pneumococcal vaccination enhances the killing activity of serum in both HTLV-II–infected and uninfected persons, and this activity is likely critical for clearance of the organism by phagocytes [8].

In conclusion, we have found that persons infected with HTLV-II develop robust humoral responses to potentially protective polysaccharide and protein vaccines. That HTLV-II–infected persons have the ability to respond to immunization is significant, because they are at high risk for disease and may be a promising target for the receipt of such common vaccines as pneumococcal polysaccharides and influenza proteins to prevent infection. Further research is needed to ascertain whether an immunological basis underlies the epidemiological link between HTLV-II infection and increased incidences of infectious diseases, including of pneumonia.

Acknowledgment
We thank Jessica Li for providing technical assistance.

References