

Letter to the Editor

Dominance of human innate immune responses in primary *Francisella tularensis* live vaccine strain vaccination

To the Editor:

Francisella tularensis is the etiologic agent of the zoonotic disease, tularemia. An inoculum as small as 10 bacteria can cause a flulike disease with substantial morbidity and mortality among infected humans.^{1,2} Human tularemia presents in ulceroglandular, oculoglandular, oropharyngeal, pneumonic, and septic forms.^{1,3} Rapid administration of antibiotics prevents mortality in the majority of human cases if exposure doses are low and nonaerosol.^{2,4} Without early diagnosis and administration of antibiotics, high-dose aerosol exposure progresses rapidly to life-threatening pleuropneumonitis and systemic infection.³ The relative abundance of *F. tularensis* in nature and the relative ease with which it may be administered raise concerns over its exploitation as a biothreat agent.^{1,3,4}

In the 1950s, an attenuated strain of *F. tularensis* was developed into an Investigational New Drug status live vaccine (live vaccine strain [LVS]) administered by intradermal scarification.^{1,5,6} LVS vaccination has significantly lowered reports of laboratory-acquired tularemia, although the mechanism of protection (humoral or cell-mediated) is still unclear.^{1,3,7} Microagglutination assays performed at 28 days postvaccination (indicating anti-*F. tularensis* IgG and IgM) are the clinical standard for gauging successful vaccination yet show poor correlation with specific, vigorous lymphocyte responses in LVS-vaccinated and naturally infected humans.⁸ Human anti-*F. tularensis* immune serum resulting from vaccination with LVS is protective only against strains of reduced virulence, yet immunospecific and long-lasting cell-mediated immunity is the key to protection.^{5,7,9} Substantial data suggest that cell-mediated immunity may be more important than humoral immunity for providing long-lasting immunity against virulent strains of *F. tularensis*.^{5,7,9} Therefore, we examined human immune responses to LVS vaccination to establish early cellular correlates of LVS-mediated protection predictive of successful vaccine outcomes.

Volunteers were recruited from US Army Medical Research Institute of Infectious Diseases (USAMRIID) personnel at risk of laboratory exposure to *F. tularensis*. A minimal risk protocol to collect peripheral blood samples was approved by institutional review boards at the USAMRIID (Human Use Committee FY04-16). Donors provided informed consent and met eligibility criteria. Six healthy adults (4 males and 2 females, 22-54 years old) received a primary LVS vaccination and donated peripheral blood prevaccination and postvaccination. Mononuclear cells were purified by Ficoll gradients and assessed for changes in immune cell populations using flow cytometry and quadruple stained using directly conjugated mAbs (BD-Immunocytometry Systems and BD-Pharmingen, La Jolla, Calif). Cytometric bead

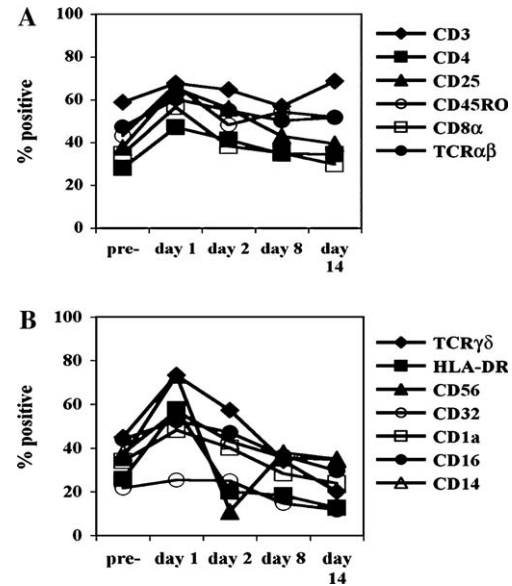


FIG 1. Cellularity changes in acquired and innate immune response to LVS vaccination. Peripheral blood was monitored for changes in acquired (A) and innate (B) immune cell surface markers over the course of several days prevaccination and postvaccination (as indicated). Symbols represent the mean percentage positive cells with $n = 6$.

array analysis was performed on serum samples (BD-Pharmingen). Analysis was performed using FlowJo (TreeStar, Inc, Ashland, Ore) and GraphPad Prism (GraphPad Prism Software, San Diego, Calif). All 6 subjects were immunologically naive before vaccination. All vaccinations had positive responses as indicated by initial formation of a small pustule/papule and subsequent ulceration.

Cell surface analysis of mononuclear cells revealed bias toward activation of innate versus acquired immune system components. The greatest changes in cellularity occurred on day +1 ($P < .001$ for day +1 versus all other days), a time frame consistent with innate immunity, but too short for unprimed acquired responses (Fig 1, A and B). When acquired immune system cells ($CD3^+$, $CD4^+$, $CD8^+$, T-cell receptor [TCR] $\alpha\beta^+$, $CD45RO^+$) were analyzed over all time points, only $CD4^+$ and $CD8^+$ cells were significantly changed at day +1 ($P < .05$ and $P < .01$, respectively).

Although $CD4^+$ and $CD8^+$ cells increased significantly on day +1 (Fig 1, A), these cells were not conventional $TCR\alpha\beta^+$ T cells, but rather $TCR\gamma\delta^+$ T cells ($CD8^+/\gamma\delta TCR^+$), natural killer (NK) T cells ($CD56^+/CD8^+$), and monocytes ($CD4^+/CD14^+$) whose kinetics mirrored innate immune responses (Fig 1, B). When innate immune system components ($CD56^+$, $CD1a^+$, $TCR\gamma\delta^+$, human leukocyte antigen [HLA]-DR $^+$, $CD16^+$, $CD14^+$) were tracked over the course of the vaccination, NK cells, $\gamma\delta$ T cells, monocytes, granulocytes, and dendritic cells showed considerable changes in cellularity on day +1. Dramatic increases in $\gamma\delta$ T cells (Fig 2, A) and NK cells

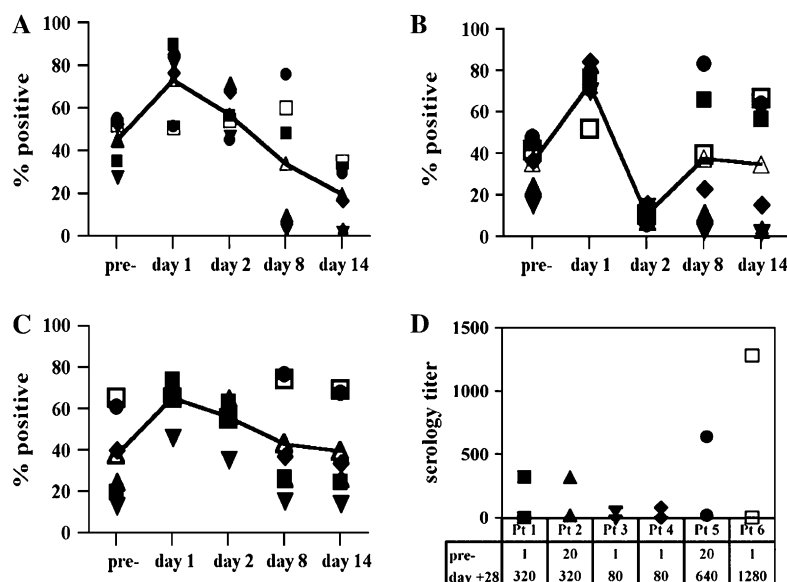


FIG 2. LVS vaccination promotes strong innate immune responses. PBMCs were analyzed for cell surface markers indicating $\gamma\delta$ T cells (A), NK cells (B), and competence for proliferation, CD25 (C). Data represent individual patient values for each marker with a line connecting the average value for all 6 patients over time. D, Serologic titers for individual patients, prevaccination and postvaccination. Symbols represent individual patient titers and are consistent with individual cellular responses indicated (A-C).

(Fig 2, B) were noted on day +1 ($P < .05$ and $P < .01$, respectively), with NK cells having the most prominent changes. LVS vaccination induced a strong proliferative signal for innate lymphocytes as measured by the upregulation of the IL-2 receptor high-affinity α chain, CD25 (Fig 2, C), whose induction is linked with progression into cell cycle.¹⁰ CD25 upregulation was highest on day +1 for all donors ($P < .01$) and was noted for NKT, $\gamma\delta$ T, and NK cells. No similar correlations were seen between CD25 and TCR $\alpha\beta^+$ -CD4 $^+$ or TCR $\alpha\beta^+$ -CD8 $^+$ T cells, even at time points associated with primary acquired immune responses, days +8 to +14.

Our data strongly suggest that cellularity changes after human LVS vaccination strongly parallel innate immune system kinetics. These data are in accord with the murine LVS model in which mice deficient in T cells are still able to resist lethal LVS challenge for 3 to 4 weeks.⁵ This protection can be traced to proinflammatory cytokine production by innate immune components, chiefly NK and NKT cells.⁵ Although serum cytokine levels in our study were below the limit of detection (<20 pg/mL), the upregulation of the high-affinity IL-2 receptor chain (CD25) may indicate that a proinflammatory T_H1 -type response was induced by LVS vaccination. All 6 of the subjects had positive anti-LVS titers by +28 days, yet there was as much as a 16-fold difference in positive microagglutination titers (Fig 2, D). Cellular responses at day +1 and +2 showed a much tighter cluster across all 6 subjects (less than 2-fold change between subjects), consistent with data suggesting the critical importance of cell-mediated immunity in long-term anti-*F. tularensis* protection.^{5,7,9} Surprisingly, postvaccination titer bore no resemblance to the cellular immune response described (Fig 2, D).

Combined with data suggesting cell-mediated responses are most critical to anti-*F. tularensis* protective immunity, the question of the role of humoral immunity remains unanswered.^{5,7,9} Most importantly, our data point to cellular correlates of protection predictive of positive vaccine outcomes as early as 24 hours postinfection. Future studies to determine the gene-level responses to human LVS vaccination are underway in our laboratory.

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