O antigen limits complement protein C3 deposition on the surface of types A and B F *tularensis* and enables them to resist complement-mediated lysis (13).

Type B (live vaccine strain, LVS) and type A (Schu S4) strains differ in virulence. One potential mechanism of Schu S4 virulence is the active suppression of the pulmonary immune response, in part through the induction of TGF-β, which allows Schu S4 to evade detection and to actively suppress the in vivo responses to secondary stimuli, such as LPS (14). Undoubtedly, additional virulence genes will be identified in Schu S4.

In animal studies, the host response is a major factor in the morbidity and mortality of tularemia. Neutrophils appear to play a role in host defenses against *F. tularensis* infection inasmuch as mice unable to recruit these cells into infectious foci rapidly die from lower doses of LVS (15). *Francisella tularensis* also blocks the respiratory burst within polymorphonuclear leukocytes (PMNs) (16). Matrix metalloprotein 9, a neutrophilic protein, increases host susceptibility and lack of this protein renders mice protected against Schu S4 (17). Infection with *F. tularensis* ssp. *holarctica* induces massive expansion of circulating γΔ T cells whose function is unknown (18).

MECHANISMS OF HOST PROTECTION

Antibodies

In mice, antibodies alone can protect against lethal systemic challenge with low virulence strains, but not against aerosol challenge with a fully virulent type B strain or against either systemic or aerosol challenge with type A strains. Human subjects immunized with the LVS mount an antibody response to the LVS LPS and to many protein antigens, although no correlation was found between levels of agglutinating antibody to Foshay or LVS vaccines and protection.

Passive immunization with serum collected from mice immunized with either a heat-killed preparation of *F. tularensis* LVS or an O-antigen deficient mutant yielded similar protection against homologous live LVS challenge. These data suggest that antibodies alone can confer protection against LVS challenge, and that these protective antibodies are not dependent on anti-O-specific antibodies (19). The protective role of serum antibodies against *Francisella tularensis* was also demonstrated when immune serum was passively administered to naïve mice before respiratory challenge with LVS. The protective effect of this serum prophylaxis (100%) was independent of complement, but required interferon gamma (IFN-γ). Since severe combined immunodeficiency (SCID) mice were not protected by passive antibody transfer, cooperation between humoral and cellular immune responses was considered necessary for sterilizing immunity to *F. tularensis*, and that T cell, not NK cells, might be the source of this IFN-γ (20).

Protection against respiratory LVS tularemia by intranasal administration of inactivated *F. tularensis* LVS required exogenous IL-12 as an adjuvant. Interestingly, mice genetically deficient in immunoglobulin A expression did not survive. Thus, IgA-mediated protection may have a role in protection against pulmonary tularemia following mucosal immunization (21).

Adaptive Immune Response

Given the relatively recent development in our understanding of the adaptive immune response, assays of cellular immune responses were not performed in early human vaccine studies. The Soviets considered the duration of immunity with their vaccines to be five years, although more recent data with the LVS suggest that cell-mediated immunity to LVS persists for at least 25 years (22). While it is assumed that the adaptive immune responses elicited by LVS are primarily responsible for the protection, there is little direct evidence. It has been shown that long-lasting specific $CD4^+$ and $CD8^+$ T-cell immunity and proliferation of natural killer (NK) and monocytes developed against protein antigens after LVS immunization. Further, protection afforded to mice by LVS against type A strains can be abrogated by depletion of either $CD4^+$, $CD8^+$ T cells or by neutralization of IFN-γ prior to challenge (23). This remains the best evidence of cellular immune response as being required for efficacy of LVS in mice; however, one cannot disregard the possibilities that antibodies to other (protein) antigens may play a role.

VACCINES

While the relatively limited burden of naturally acquired human tularemia disease would not argue for the need for a vaccine, the previous use and deployment of *F. tularensis* as a bioweapon in the 1940s (Soviets, Americans, and Japanese in Unit 731 before World War II) has led to the development of vaccines to provide a countermeasure. During World War II, tens of thousands of soldiers on the eastern front were infected with tularemia, perhaps through its intentional release. While Soviet soldiers were said to receive mass aerosol inoculations, the major impetus for vaccine development in the United States was to protect laboratory workers engaged in biological warfare programs. Although Francis himself developed infection at least on three occasions, tularemia infection in man is generally considered to protect against subsequent infection. In experimental systems, protection is afforded by natural infection or by live attenuated strains, but little by killed vaccines (24).

Killed Vaccine

The initial vaccines developed in the West were heat- and formalin-killed, but these were highly reactogenic and poorly immunogenic, perhaps due to alteration of the *F. tularensis* antigens. Dr Lee Foshay prepared a killed whole-cell vaccine by acid/phenol extraction that was not highly efficacious against challenge of mice and nonhuman primates with Schu S4, a protype type A strain; however, studies in humans demonstrated that it both reduced the number of infections and if infected, modified the course of disease (25). This vaccine was administered to several thousand individuals, including laboratory workers at high risk of infection, and was better tolerated than previous killed vaccines (26).

Live Attenuated Vaccine

The Foshay vaccine modulated ulceroglandular and typhoidal tularemia, but did not protect against type A infection. The failure of killed vaccines to induce solid protection has been attributed to its failure to induce a potent cellular immune response, which is considered necessary for protection against virulent type A infection. The only vaccines in wide use against bacteria that like *F. tularensis* are intracellular are live attenuated organisms. These include BCG (Bacillus Calmette-Guérin) against tuberculosis and strain Ty21 and live oral vaccine against typhoid fever; both these live vaccines induce potent cellular immune responses (8).