phagocytosis of the bacteria (29,35,56,57), leading one to conclude that the humoral immune response is critical for protection against plague.

Although this is undoubtedly true, other studies paint a more complex picture of plague pathogenesis and suggest that the ability of cellular immune responses to clear intracellularly replicating bacteria is also an important factor. For example, work by Cavanaugh and Randall (56) confirmed and extended prior findings by demonstrating that plague bacilli expressing F1 and LcrV are indeed resistant to phagocytosis by PMNs and monocytes. In addition, bacteria only expressing LcrV were resistant to phagocytosis by PMNs but were readily ingested by monocytes, while bacteria expressing neither F1 nor LcrV were phagocytosed by both cell types. However, they showed that while PMNs killed ingested bacteria, monocytes failed to do so and, in fact, bacteria within monocytes were able to replicate extensively. Following growth in monocytes, the released bacteria expressed both F1 and LcrV. Together, these results suggest that monocytes may constitute an initial protective niche for *Y. pestis*, thereby allowing the bacteria time to express F1 and LcrV. Consistent with this view, Lukaszewski et al. (69) demonstrated that, following subcutaneous delivery of *Y. pestis* grown at 28°C, bacteria were able to survive and replicate within splenic macrophages until the later stages of infection, at which time extracellular bacteria were observed. Likewise, Finegold (65) reported finding intracellular bacteria even in late stages of infection following aerosol exposure of rhesus monkeys using bacteria cultured at 28°C. However, Lukazewski et al. (69) demonstrated that pretreatment of macrophages with TNF-α and IFN-γ restricted intracellular replication. In addition, a prior study by Nakajima and Brubaker (66) showed that mice dosed with TNF-α and IFN-γ were protected against intravenous challenge with a non-pigmented (*pgm*-) strain of *Y. pestis*.

The aforementioned studies suggest that Th1 type cytokine responses are an important component in the immune response against plague. These findings were extended by Smiley and coworkers who demonstrated that vaccination of mice with an attenuated (*pgm*-) strain of *Y. pestis* primes T cells that provide passive protection against intranasal challenge with the same strain (70). Similarly, vaccination of B cell–deficient μMT mice with an attenuated *Y. pestis* strain also conferred protection against challenge (71). While clearly demonstrating a role for T cells in the immune response against plague, the results should be interpreted with some caution as Pujol et al. (72) demonstrated that the *ripA* gene, which is encoded within the *pgm* locus, suppresses nitric oxide production by IFN-γ-activated macrophages and is required for intracellular replication. This suggests that pigmentation-negative *Y. pestis* strains may therefore be more sensitive to cell-mediated immune responses than wild-type strains. In addition, it should be pointed out that passive transfer of T cells has not been shown to confer protection against a wild-type strain when administered via the aerosol route. Nevertheless, these studies suggest that while antibodies against F1 and LcrV promote phagocytosis, cytokine production by T cells promotes the killing of bacteria that have been ingested by macrophages.

KILLED WHOLE-CELL VACCINES

Despite the inherent dangers of *Y. pestis*, no suitable vaccine exists (6). A killed whole-cell vaccine was first developed by Haffkine in 1897. Although the vaccine was reported to be effective against bubonic plague, it was highly reactogenic (73). Symptoms included pain, swelling, erythema, and regional lympadenopathy. For these reasons, such vaccines largely fell out of favor until Meyer and colleagues developed a less reactogenic killed whole-cell vaccine (eventually called Plague Vaccine, USP), which served as a vaccine for the U.S. military since the 1940s. Military personnel in WWII and Vietnam who were vaccinated did not contract plague (74). Despite these results, Plague Vaccine, USP still retained unwanted reactogenicity, required multiple boosts, and is no longer currently manufactured (6). In addition to their unwanted side effects, the chief limitation of killed whole-cell vaccines is their inability to protect against pneumonic plague in animals (75) and humans (58).

LIVE-ATTENUATED VACCINES

The first, and so far only, live bacterial plague vaccines to be used in humans are the attenuated *Y. pestis* vaccine strains EV (and its derivatives) and Tjiwidej (74). These strains were widely used in plague pandemic regions of Africa and Asia and vaccination programs using these strains were shown to reduce the incidence of plague in these areas. The EV76 strain, which lacks the 102 kb chromosomal pigmentation (*pgm*) locus, confers protection against both bubonic and pneumonic plague (76,77). However, this strain, while essentially avirulent by the subcutaneous route in rodents, is lethal to nonhuman primates at moderate doses and causes significant side effects in humans (76). More recently, a *Y. pestis* strain that lacks both the Pla protease and the *pgm* locus, and which is less virulent than the EV76 strain, was shown to induce a humoral immune response against F1 in African green monkeys following aerosol delivery (78). Whether this immune response is protective is not yet known. Other strategies to generate live plague vaccines have involved the introduction of a *lpxM* mutation into the EV strain background, thereby generating a strain that synthesizes a less toxic penta-acylated LPS (79), the creation of a DNA adenine methylase *dam* mutant (80), and deletion of the type III effector YopH (81) from wild-type *Y. pestis* strains. While these strains were immunogenic and conferred protection against subsequent challenge with a wild-type strain, the development of live attenuated *Y. pestis* strains that are safe and immunogenic in humans faces a high hurdle.

SUBUNIT VACCINES

It has long been established that immunization with F1 purified from *Y. pestis* protects animals against bubonic (82) and pneumonic (83) plague. More recently, purified recombinant F1 was shown to confer protection against pneumonic plague in mice (84). However, the F1 antigen is dispensable for virulence (58,59,61–63). Therefore, vaccines based solely upon F1 will not confer protection against almost fully virulent F1-negative strains.

For this reason, researchers have also developed LcrV-based subunit vaccines, as LcrV is an indispensable virulence determinant (30,31) and has long been known to be a protective antigen (85). Many studies demonstrated that passive immunization with antibodies directed against LcrV confers protection against plague (32–34,86). Similarly, protection is observed following immunization with purified recombinant LcrV (87,88). Although LcrV is an indispensable virulence determinant, sequence analyses of LcrV from *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* revealed a region of LcrV whose sequence is variable, particularly in O:8 serotype strains