of *Y. enterocolitica*. Antibodies generated against the O:8 LcrV do not provide substantial passive protection against strains expressing other LcrV variants (89).

For this reason, researchers have focused on generating subunit vaccines that consist of both F1 and LcrV, which should be more difficult to circumvent. In fact, such vaccines provide enhanced protection against plague (90). Importantly, immunization with an admixed preparation of F1 and LcrV protects against aerosol challenge (75,91). The combined IgG1 titer to F1 and LcrV correlates with protection against plague in mice (92). Similarly, immunization with an F1-LcrV fusion protein protects mice against challenge with F1-positive and F1-negative strains (93,94). The F1-LcrV fusion protein also conferred high levels of protection in cynomolgus macaques against aerosolized *Y. pestis*. However, only low levels of protection were observed in African green monkeys, possibly because of more variable immune responses against LcrV (95). Antibody titers to the F1-LcrV fusion protein did not correlate with protection in African green monkeys. For this reason, researchers developed an in vitro system to identify correlates of protection. These models take advantage of the fact that pathogenic yersinae induce a cytotoxic effect in macrophages, as evidenced by changes in cellular morphology, the production of the apoptosis-specific enzyme caspase-3 and release of the cytosolic enzyme lactic acid dehyrdrogenase. Antibodies against LcrV neutralize these cytotoxic effects by blocking the injection of type III effectors (23,29,35), and the neutralizing activity of anti-LcrV antibodies correlates with protection in both mice (96,97) and nonhuman primates (98).

Recently, the combined F1 + LcrV subunit vaccine was tested in a phase I clinical trial (99). Groups of individuals were immunized on days 1 and 21, with either 5, 10, 20, or 40 μg of F1 and LcrV (admixed in alum in a 2:1 molar ratio). Antibodies to both F1 and LcrV were produced within two weeks of the first dose and increased after the booster dose on day 21, although there was a large variation in antibody responses between individuals within each dose group. Passive transfer of protective immunity correlated with total combined IgG titers to F1 and LcrV on day 21. Importantly, this subunit vaccine was not associated with adverse side effects. However, IgG titers declined by day 70 in all dose groups.

NEW APPROACHES

Given the relatively low and variable immune responses observed with the F1 + LcrV subunit vaccine, much attention has been focused recently on developing new methods to generate more robust immune responses against these antigens. These include the use of DNA vaccines (53,99–101), the use of viral vectors for antigen delivery (100,102), encapsulation in microspheres (103,104), the use of adjuvants other than alum (105–107), different routes of administration (103,107), heterologous prime-boost vaccination regimens (108), expression of F1 and LcrV in attenuated *Salmonella* vaccine vectors (109–112), and use of attenuated strains of the closely related enteric pathogen *Y. pseudotuberculosis* (113–115). While potentially promising, many of these approaches have serious limitations. For instance, DNA vaccines often require multiple immunizations (sometimes using ''Gene Gun'' technology) and have yet to show promising results in humans. Viral vectors and other forms of intranasal delivery have possible safety concerns. Testing new adjuvants will require substantial further research. Many of the aforementioned *Salmonella* vaccine vectors have yet to be tested in humans. Finally, attenuated *Y. pseudotuberculosis* strains run the risk of causing post-infectious sequelae such as reactive arthritis (116), particularly in HLA-B27positive individuals (117).

CHARACTERISTICS OF AN IDEAL PLAGUE VACCINE

Clearly, there remains an immediate need to develop new and improved plague vaccines. One can envision several characteristics of the ideal plague vaccine regimen:

A strong broad protective immune response—The ideal vaccine regimen must induce a very high level of protection. Biological attack could subject at least some victims to levels of exposure far higher than those seen after natural infection. Attacks are likely to be via the respiratory route, and thus some component of mucosal immunity may be helpful; in a pneumonic plague outbreak, some victims succumbed with mucosal and not pulmonary pathology (8). Cell-mediated immunity to the intracellular *Y. pestis* should also be beneficial; mice deficient in antibody responses can be protected by vaccination (71). Vaccines should engender a rapid response and the potential to augment the response still further by administering a booster dose.

Ease of rapid administration—Implementation of a plague vaccine regimen will likely require either vaccination of large numbers of individuals or, in the event of release, very rapid vaccination of a small but dense population. In either case, ease of administration (including a convenient supply chain) would be a great benefit. The optimal vaccine should be deliverable by personnel with limited formal expertise.

Rapid and cost-effective production—Since the government will likely need to produce and maintain large stocks of vaccines against several agents, each vaccine should be inexpensive to produce and should have a reasonably long shelf life. Multivalency, protection against more than one infection in a single vaccine, would be advantageous. Rapid production would also be helpful, so that the vaccine can be made quickly in response to a manifest threat. Perhaps an even greater advantage would be if the vaccine provided some positive externality, such as protection against another infection whose prevention appealed to an established and predictable market.

High degree of safety—It is likely that in the event of release, or prior thereto, we will need to vaccinate large numbers of people who will probably never actually encounter the agent. Therefore, it will be important to assure that the vaccine does not cause a greater degree of cumulative morbidity than the agent it is designed to thwart.

THE PROMISE OF PRIME-BOOST VACCINATION

As described above, subunit vaccines are in advanced stages of development. However, we feel that the subunit vaccines currently under evaluation will not provide optimal protection against these agents. In addition, they will not engender mucosal or cellular immunity, nor are they convenient to administer rapidly or in multiple-dose regimens. Live-attenuated mucosal vaccines have all of the advantages sought in the ideal biodefense vaccine, except that the magnitude of the immune response has not yet reached the level required for biodefense. Thus, there exists the possibility of combining two of the above