Boosting Vaccine for Tuberculosis

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An effective new vaccine for the control of tuberculosis is badly needed. While current research attempts to improve vaccination are concentrating on new prophylactic or immunotherapeutic vaccines, virtually no emphasis has been placed on boosting individuals already inoculated with Mycobacterium bovis BCG. It is shown here that mice vaccinated with BCG gradually lose their capacity to resist an aerosol challenge infection as they age. However, if these mice are inoculated with the 30-kDa mycolyl transferase A protein in midlife, after challenge when aged they express levels of protection in the lungs comparable to those of young mice, associated with minimal pathological damage.

Currently available epidemiologic data indicate that disease caused by the facultative intracellular bacterial parasite Mycobacterium tuberculosis remains a serious global problem, with around 8 million new cases per year, and there is recent disturbing evidence that the death rate may be increasing (4, 7, 14, 15). For several decades the Mycobacterium bovis-derived bacillus Calmette-Guérin (BCG) has been the only widely used vaccine for tuberculosis, and accumulating data from clinical trials and subsequent meta-analysis (5, 18) have tended to reveal its general ineffectiveness in adults (including those vaccinated with BCG as young children). As a result, many laboratories are now involved in a major effort to develop a new vaccine (12, 13), with virtually all efforts directed towards discovering new candidate vaccines that can be used in a prophylactic or immunotherapeutic mode (1, 8, 11).

The mechanism underlying the gradual loss of effectiveness of BCG as the neonatally inoculated individual reaches 10 to 15 years of age is poorly understood. One possible assumption is that memory immunity generated by BCG has disappeared and the individual is now equivalent to a naive host who can be vaccinated with a new candidate vaccine designed to induce primary immunity. An alternate possibility is that memory immunity slowly declines but can be recovered by boosting if a candidate antigen that can be specifically recognized by this immunity is reintroduced. The results of this study support the latter contention.

Several laboratories have shown that proteins found in the culture filtrates of M. tuberculosis are highly immunogenic and have promise as candidate vaccines (12). A component of this pool of proteins is the mycolyl transferase A (Ag85A) (3), and we have previously demonstrated that the largest proportion of CD4 T cells accumulate in the lungs of memory-immune mice after challenge infection recognize this antigen (6). Thus, to determine if Ag85A has the potential to boost existing memory immunity in BCG-vaccinated mice, these animals were boosted twice at 9 and 15 months of age and then challenged with virulent M. tuberculosis when elderly. As shown here, this procedure restored the capacity of these mice to express resistance to this infection.

Female C57BL/6J mice were purchased from Jackson Laboratories, Bar Harbor, Maine. They were kept under barrier conditions for the duration of the experiments. Because aged mice of this strain periodically develop tumors, each was checked by autopsy after euthanasia and positive animals were discarded from the study. Mice were vaccinated subcutaneously with 106 M. bovis BCG strain Pasteur organisms and challenged by aerosol exposure with M. tuberculosis strain H37Rv using an inhalation device (Glas-Col, Terre Haute, Ind.) calibrated to deliver about 50 bacteria into the lungs. Both organisms were grown to mid-log phase in Proskauer-Beck medium containing 0.02% Tween 80 and frozen at −70°C until used. Bacterial counts in the lung were determined by plating serial dilutions of individual whole-organ homogenates on nutrient 7H11 agar and counting bacterial colony formation after 3 weeks of incubation at 37°C in humidified air.

Culture filtrate proteins were purified from mid-log-phase cultures of M. tuberculosis H37Rv by ammonium sulfate precipitation, followed by purification of fractions containing the Ag85A molecule by hydrophobic interaction chromatography as previously described (3). Prior to inoculation these materials were emulsified in monophosphoryl lipid A adjuvant (Corixa Ribi, Hamilton, Mont.) solubilized in 0.02% triethanolamine plus 100 μg of recombinant interleukin-2 cytokine (Chiron, Emeryville, Calif.). Bacterial counts were determined as described above at 30 days after challenge. Fixed tissue sections were stained with hematoxylin and eosin and read by a pathologist without prior knowledge of the treatment groups. Differences in bacterial load between groups were tested by analysis of variance, with probability values of below 0.05 being taken as significant.

The protection conferred against an aerosol challenge infection with virulent M. tuberculosis in mice of increasing age is shown in Fig. 1. In this experiment mice were vaccinated when they were 6 to 8 weeks of age and then challenged at various ages thereafter. In mice 3 and 12 months of age, a 10-fold reduction in bacterial load in the lungs 30 days after challenge was consistently seen. In their second year of life (this mouse
then challenged by aerosol with *M. tuberculosis*; studies had shown that the response was in decline. The Ag85A-boosted group had statistically lower lung bacterial counts than the saline-treated controls (P > 0.001). Data shown are means ± standard errors of the means; n = 4.

**FIG. 2.** Evidence that vaccination followed by boosting with Ag85A improves resistance to aerosol challenge compared to BCG vaccination alone. Mice were inoculated with BCG as described in the legend to Fig. 1 at 8 weeks of age; controls were injected with saline. At 9 and 15 months of age they were boosted with either culture filtrate protein (CFP) or Ag85A in the monophosphoryl lipid A–recombinant interleukin-2 vehicle and then challenged by aerosol at 20 months of age. Only the Ag85A-boosted group had statistically lower lung bacterial counts than the saline-treated controls (P > 0.001). Data shown are means ± standard errors of the means; n = 4.
exceed that conferred by BCG, and more importantly, there are no data yet to show that a sustained long-lived state of memory immunity can be established using individual proteins or mixtures of proteins. In our own experience, Ag85A can induce modest levels of protection in mice challenged 30 days later (2). In guinea pigs no effect on the bacterial load is observed, although some modest pathological benefit in terms of some degree of lymphocytic granuloma formation in lungs coupled with reasonable long-term survival can be seen (unpublished observations); this, however, does not compare to the benefit from BCG, with which the bacterial load is reduced 2 to 3 log units and the animals live for much of their normal life span. A much better result is obtained if the Ag85A is delivered in the form of a DNA vaccine, with which good long-term survival can be seen (9). Again, however, this strategy does not appreciably reduce the bacterial load in the sensitive guinea pig model.

It is unknown why Ag85A cannot prime test animals to the degree achieved by BCG, and this underscores our present inability to induce a strong TH1 primary and long-lived memory response to nonliving vaccines similar to levels attained using BCG. A huge limitation is the paucity of vaccine adjuvants; we and others have worked with relatively mild adjuvants such as monophosphoryl lipid A and dimethyl-dioctadecyl ammonium bromide primarily because we need a material that could be safely given to people. These alone are probably not enough to induce long-lived TH1 immunity, but they may be enough to allow boosting with antigen if a TH1 state of immunity is already imprinted in the host in the form of specific immunological memory.

Finally, while there is a desperate need to develop new tuberculosis vaccines to deal with the global emergency in general, in more developed countries tuberculosis continues to be relatively common in elderly people (16, 17). In the United States, for instance, people over the age of 65 have for some time now represented the fastest-growing segment of the over-

FIG. 3. Representative photomicrographs of vaccine-boosted mice following aerosol infection with M. tuberculosis. All plates were stained with hematoxylin and eosin. (A) Lung adjacent to a bronchiole (arrowhead) from a mouse from the BCG vaccination group. Alveolar septa containing epithelioid macrophages and infiltrates of neutrophils, often forming small aggregates, are evident (arrows). (B) Lung adjacent to a bronchiole (arrowhead) from a mouse from the BCG-Ag85 vaccination group. The alveolar septa show evidence of influx by epithelioid macrophages, whereas neutrophils are scattered and rare (arrows). (C) Higher magnification of lung from a mouse from the BCG vaccination group. Arrows depict an aggregate or pocket of neutrophils within an alveolar septa. (D) Higher magnification of lung from a mouse from the BCG-Ag85 vaccination group. The septa are thickened by an influx of macrophages. Neutrophils are rare; the arrow indicates a solitary neutrophil. Bars, 10 μm.
all population. While primary tuberculosis sometimes occurs in these individuals, the majority of cases are thought to be due to reactivation of latent disease acquired many decades earlier (17). In view of this, the vaccine strategy espoused here may also have applications to prevention of reactivation tuberculosis in the elderly.

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REFERENCES