ANIMAL MODEL OF HUMAN DISEASE

Pulmonary Tuberculosis

Animal Model: Experimental Airborne Tuberculosis in the Guinea Pig

Contributed by: D. W. Smith, PhD, and G. E. Harding, PhD, Department of Medical Microbiology, University of Wisconsin, Madison, Wisconsin 53706.

Biologic Features

Tuberculosis remains at or near the top of the list of public health priorities for many of the developing countries of the world. One important control measure, the use of Bacille-Calmette-Guérin (BCG) vaccine, has been shown to be 60 to 80% effective in reducing the occurrence of pulmonary tuberculosis.[1] BCG is infrequently used in the United States, even in high-risk populations, in part because of uncertainty about the protective potency of currently available vaccines and also because vaccination with BCG causes tuberculin conversion.[2] The mechanism by which BCG vaccination protects against tuberculosis is not known; therefore, an animal model of tuberculosis should contribute to our understanding of this mechanism, leading to more effective vaccines, perhaps ones that do not induce long-lasting tuberculin sensitivity.[3]

Many animal models of tuberculosis have been developed. However, they disagree on the assignment of potency, and the specific independent and dependent variables in the animal test system clearly influence the apparent potency of the vaccines.[4] Until it is known through a proposed Joint Field Trial–Animal Model Assay [5] which animal models predict the potency of vaccines for man, we have used an animal model patterned as closely as possible after the conditions under which man is infected.[6]

Guinea pigs (*Cavia cobaya*) can be infected by the respiratory route with small numbers of virulent *Mycobacterium tuberculosis*. The technology enabling the reproducible implantation and retention of as few as two to three viable mycobacteria in the lungs of guinea pigs has been developed.[7] Contrasting the time course of this infection in vaccinated and nonvaccinated guinea pigs has revealed several important findings. Virulent organisms multiplied at the same rate in the lungs of vaccinated and nonvaccinated guinea pigs during the first 2 weeks after challenge;

Publication sponsored by the Registry of Comparative Pathology of the Armed Forces Institute of Pathology and supported by Public Health Service Grant RR 00301 from the Division of Research Resources, US Department of Health, Education and Welfare, under the auspices of Universities Associated for Research and Education in Pathology, Inc.

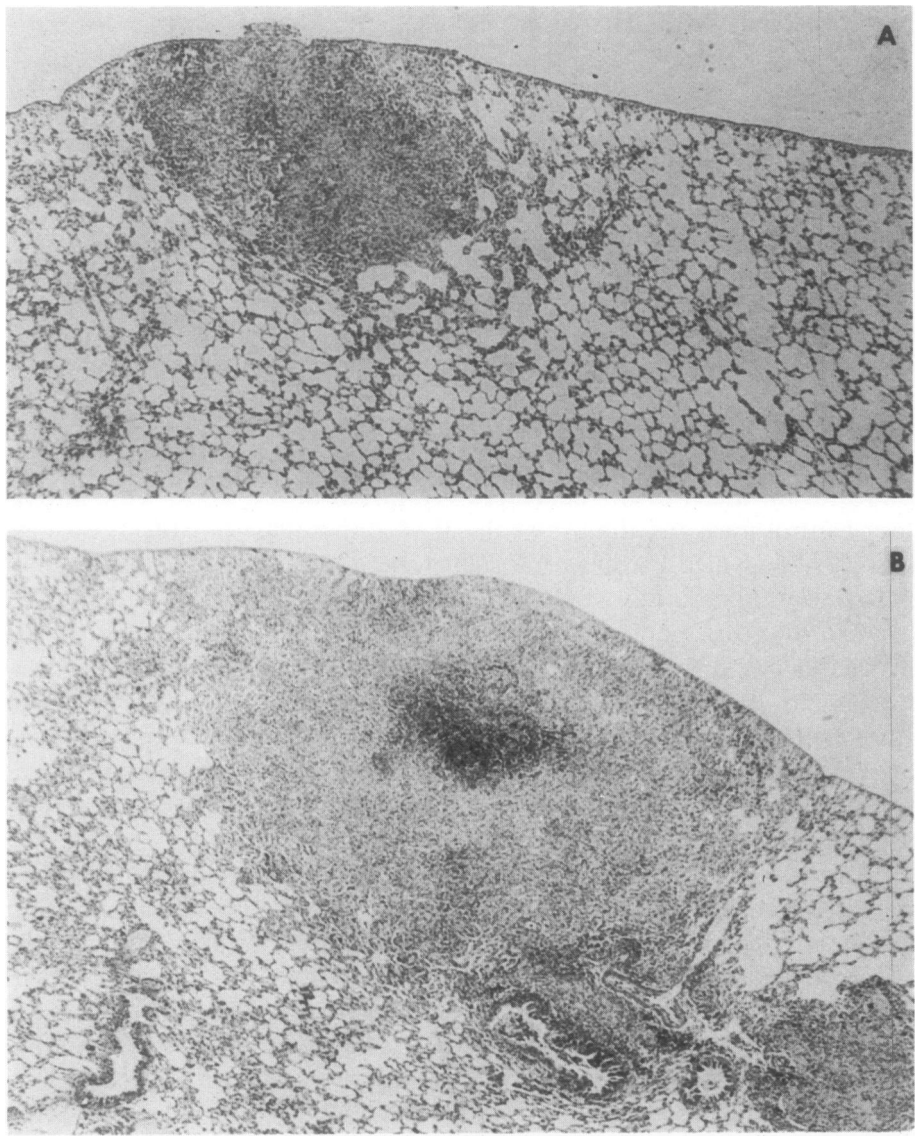

Figure 1—Primary lesions in the lungs of guinea pigs killed 24 days after inhalation of approximately three viable virulent mycobacteria. A—BCG-vaccinated animal. B—Nonvaccinated animal.

then multiplication slowed down, first in animals vaccinated with a potent BCG vaccine, then a few days later in animals given a less potent vaccine, and still later in those given a placebo at the time of vaccination.[7] The attendant tissue damage in the primary lesions of vaccinated and nonvaccinated animals killed 24 days after infection is shown in Figure 1. Cavities (Figure 2) were evident in the lungs of nonvaccinated but not vaccinated animals killed 12 to 14 weeks after aerosol challenge with two to four virulent tubercle bacilli. Another important finding, which depended on the low level of infection and the quantitative culture of each

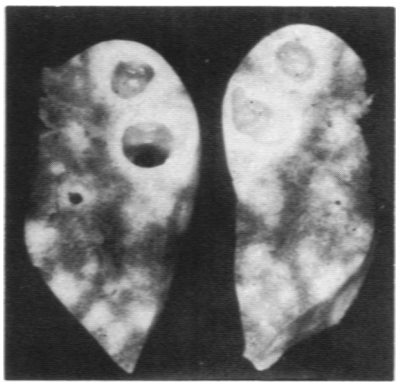

Figure 2—Cavities in cut sections of the lungs of nonvaccinated guinea pigs killed 12 weeks after challenge via the respiratory route.

of the six separate lung lobes of the animals as well as the spleens, was that about 3 weeks after low-level aerosol challenge, bacilli entered the bloodstream of the nonvaccinated infected animals and were carried back to the lung in sufficient numbers so that each lobe retained viable organisms in over 100 foci.[7] Over the course of time, the hematogenously distributed bacilli multiplied and secondary lesions developed. In contrast, this bacillemic phase of experimental airborne tuberculosis in guinea pigs was markedly reduced and retarded in BCG-vaccinated animals.[8,9] Moreover, the extent to which the bacillemia is influenced appears to be a function of the potency of the vaccine.

Comparisons With Human Disease

Tuberculosis in man is acquired mainly by inhalation of droplet nuclei containing one to three tubercle bacilli. It is believed that shortly after the primary infection, a few bacilli invariably get into the bloodstream.[10-12] In most instances this bacillemia is occult; therefore, the majority of infected persons have no detectable symptoms at the time of the hematogenous distribution. It is well accepted that cavitary tuberculosis in man usually develops at sites in the apical or subapical areas of the lung.[13] On the other hand, much evidence supports the concept that these sites are not the locations of implantation at the initiation of the infection. The bacillemia occurring early after primary infection is presumably the means by which bacilli are transported back to the lungs and other tissues. A selective influence, such as high pO_2, allows the survival of a few tubercle bacilli (a strict aerobe) in sites such as the upper regions of the lung, the kidneys, growing ends of long bones, etc., whereas they appear to be killed in other hematogenously implanted foci, presumably by the interaction of the immune response of the host and the deficient supply of available oxygen.[14]

Usefulness of the Model

Given the crucial importance of the bacillemia in the transition from tuberculosis infection to tuberculous disease in man, an animal model of

tuberculosis which permits the study of a naturally occurring bacillemia should provide an important tool for studies of the pathogenesis of tuberculosis and the mode of action of vaccines. An inexpensive, reproducible animal model in which the cavitary stages of tuberculosis occur naturally should prove useful in studies of chemotherapeutic regimens or for studies of the basic phenomena involved in caseation, liquifaction, and cavitation.

Availability

Guinea pigs are readily available from many sources. Intercurrent infections can cause serious problems, and care must be taken to ensure that the guinea pigs are free from intercurrent disease. The aerosol infection apparatus, from which our chamber was modified, is available from the Tri-R-Instruments, Co., Rockville Center, NY 11570.

References

1. BCG and Vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life: Fourth Report to the Medical Research Council by its Tuberculosis Vaccines Clinical Trial Committee. Bull WHO 46:371–385, 1972
2. Morbidity and Mortality Weekly Report, Vol 24. Atlanta, Ga., Center for Disease Control, 1975, p 69
3. Knight-Shapiro CD, Harding GE, Smith DW: Relationship of delayed-type hypersensitivity and acquired cellular resistance in experimental airborne tuberculosis. J Infect Dis 130:8–15, 1974
4. Smith DW, Wiegeshaus EH, Stark RH, Harding GE: Models for potency assay of tuberculosis vaccines. Status of Immunization in Tuberculosis in 1971: Report of a Conference on Progress to Date, Future Trends and Research Needs. Fogarty International Center Proceedings, No. 14. Bethesda, Md., 1972, pp 205–218
5. Smith DW, Harding GE: Approaches to the validation of animal test systems for assay of protective potency of BCG vaccines. J Biol Stand (In press)
6. Wiegeshaus EH, McMurray DN, Grover AA, Harding GE, Smith DW: Host-parasite relationships in experimental airborne tuberculosis. III. Relevance of microbial enumeration to acquired resistance in guinea pigs. Am Rev Resp Dis 102:422–429, 1970
7. Smith DW, McMurray DN, Wiegeshaus EH, Grover AA, Harding GE: Host-parasite relationships in experimental airborne tuberculosis. IV. Early events in the course of infection in vaccinated and nonvaccinated guinea pigs. Am Rev Resp Dis 102:937–949, 1970
8. Fok JS, Ho RS, Arora PK, Harding GE, Smith DW: Host-parasite relationships in experimental airborne tuberculosis. V. Lack of hematogenous dissemination of *Mycobacterium tuberculosis* to the lungs in animals vaccinated with Bacille-Calmette-Guérin. J Infect Dis 133:137–144, 1976
9. Smith DW, Harding GE: Influence of BCG vaccination on the bacillemic phase of experimental airborne tuberculosis in guinea pigs. Mycobacterial Infections of Zoo Animals. Washington, D.C., Smithsonian Institution Press (In press)
10. Lincoln EM, Gilbert LA, Morales SM: Chronic pulmonary tuberculosis in individuals with known previous primary tuberculosis. Dis Chest 38:473–482, 1960
11. Stead WW: The pathogenesis of pulmonary tuberculosis among older persons. Am Rev Resp Dis 91:811–822, 1965
12. Pinner M: Pulmonary Tuberculosis in the Adult: Its Fundamental Aspects. Springfield, Ill., Charles C Thomas, Publisher, 1945, p 175
13. Sweany HC, Cook CE, Kegerreis R: A study of the position of primary cavities in pulmonary tuberculosis. Am Rev Tuberc 24:558–582, 1931
14. Stead WW: The natural history of tuberculosis in man.[4] pp 13–18