GUINEA PIG ALVEOLAR MACROPHAGES PROBABLY KILL M. TUBERCULOSIS H37Rv AND H37Ra IN VIVO BY PRODUCING HYDROGEN PEROXIDE

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The major phagocytic cell involved in host resistance to Mycobacterium tuberculosis infection is probably the alveolar macrophage. It seems likely that macrophages, at least in the immune animal, can kill M. tuberculosis (1) and this might involve the production of hydrogen peroxide ($H_2O_2$) by the macrophages. Macrophages release $H_2O_2$ in vitro when exposed to phagocytic stimuli or certain soluble agents that perturb the plasma membrane (8,9). $H_2O_2$ can kill M. tuberculosis and resistance to $H_2O_2$ in vitro correlates with high virulence in the guinea pig (11,5).

To assess the role of macrophage $H_2O_2$ in killing M. tuberculosis in guinea pig lungs we have compared the fate of parent M. tuberculosis strains and $H_2O_2$-susceptible variant strains during the first 6 days after i.v. infection of normal and BCG-vaccinated guinea pigs. In parallel we have examined the $H_2O_2$ releasing capacity of the macrophages recoverable by lavage from the infected lungs. The studies on bacterial $H_2O_2$ susceptibility and viability in the lung formed part of a recent detailed report (6).

In the basic procedure guinea pigs were vaccinated with streptomycin-resistant BCG 5 weeks (i.p., $3 \times 10^7$ colony-forming units (c.f.u.)) and 1 week (i.m., $1 \times 10^7$ c.f.u.) before challenge infection. Then normal and vaccinated animals were infected i.v. with $1 \times 10^7$ c.f.u. of one of the bacterial strains. At 3 h, 3 days and 6 days after infection counts of c.f.u. were made from whole lung homogenates or, in separate experiments, alveolar macrophages were obtained by pulmonary lavage with medium 199 for studies of $H_2O_2$ release. Macrophages that adhered to plastic
petri dishes after incubation for 1 h were rinsed and assayed for 
H₂O₂ release, which was measured fluorimetrically as horse radish 
peroxidase-dependent oxidation of p-hydroxyphenylacetic acid (2). 
Incubation was for 1 h with opsonised ³H-labelled H37Ra or for 
15 min with phorbol myristate acetate (PMA, 1 μg/0.1 ml) as a 
stimulus.

The fates of M. tuberculosis strains H37Rv, H37Ra and their 
isoniazid-resistant, catalase-negative mutants H37RvHR and H37RaHR 
in the lungs of normal and vaccinated guinea pigs are shown in 
Fig. 1. The parent strains were equally resistant and the mutant 
strains equally susceptible to H₂O₂ in vitro and in the lungs. 
The parent strains fared better than the mutants. In the normal 
animal the effect of the bacterial mutation to H₂O₂ susceptibility 
was the same in the first and second 3 day period, suggesting that 
H₂O₂ availability was the same in the two periods. In contrast, 
in the vaccinated animal the effect of H₂O₂ susceptibility was 
greater in the second period, suggesting an increasing availability 
of H₂O₂. During the first period the effect of H₂O₂ susceptibility 
was expressed equally in normal and vaccinated animals, suggesting 
equal initial availability of H₂O₂ in normal and vaccinated 
animals.

The number of cells obtained by pulmonary lavage of H37Ra– 
infected guinea pigs approximately doubled from 3 h to 6 days 
after infection, and the proportion of mononuclear cells increased 
from 57 to 92% in normal animals, and from 80 to 97% in vaccinated 
animals. The cells obtained contained 0.1% of the viable bacteria 
in the lungs at 3 h and this increased to about 2.5% by 6 days.

The capacity of the adherent mononuclear cells (macrophages) 
to release H₂O₂ with and without phagocytic stimulation in vitro 
is shown in Fig. 2. Vaccination did not affect the release of 
H₂O₂ from macrophages that were removed from the lungs 3 h after 
intravenous infection and tested in vitro with or without 
phagocytic stimulation. These observations were consistent with 
the evidence that H₂O₂-mediated killing of tubercle bacilli was 
the same in normal and vaccinated animals at this early stage of 
infection. Macrophages that were removed from vaccinated animals 
on the third and sixth days after infection released progressively 
more H₂O₂ than macrophages that were removed at 3 h. This 
increase was not seen with macrophages from the infected normal 
animals. Again, this observation was consistent with the 
evidence that H₂O₂-mediated killing of tubercle bacilli in vivo 
increased with the onset of acquired immunity. Phagocytosis of 
opsonised H37Ra in vitro enhanced H₂O₂ release. Figure 2b shows 
that the increment per bacterium taken up by the cells increased 
with time elapsed after intravenous infection, and that the 
increase in responsiveness occurred faster in vaccinated animals 
than in normal animals. The increase in responsiveness was