Mini Review

Potential complications to TB vaccine testing in animal models

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Testing of new vaccines in animal models has certain advantages and disadvantages. As we better understand the complexity of the immune response to vaccines, new information may be complicating the assessment of the efficacy of new candidate vaccines. Four possible complications are discussed here, (i) induction of Foxp3+ T cells; (ii) induction of memory T cell subsets; (iii) location of extracellular organisms in lung necrosis; and (iv) protection against isolates of high/extreme immunopathology.

Keywords: Animal model, Foxp3+ cells, Lung necrosis, TB vaccine, T cell

Introduction

Over the past two decades, there has been a concerted effort to develop new vaccines against Mycobacterium tuberculosis by boosting the existing BCG vaccine, improving it by recombinant technology, or replacing it with completely different types of both living or non-living vaccine candidates1,2.

New vaccine candidates are currently under development and to be tested in animal models, particularly, the mouse model. Testing in animal model is an obvious starting point, and the mouse model, like all models proposed so far, has certain advantages and disadvantages. These include the degree to which the immune response to vaccines can be measured, whether the pathology induced in the model has any similarity to humans, and practical issues, such as animal husbandry, containment space and cost.

In all efficacy screening models to date, the candidate vaccine is given by one of several injection routes, followed a few weeks later by challenge with a laboratory strain, H37Rv or Erdman, (usually given by aerosol). After a month, the ability of the vaccine to reduce the bacterial load in lungs is determined, often in concert with pathologic assessment and [where doable] flow cytometric analysis.

Animal model development has not been static even in the two most commonly used animals, mouse and guinea pig. This includes an appreciation of complexity of immune response to vaccine and challenge in the mouse1,3 and application of new technologies, such as flow cytometry4,5, imaging6, laser capture microscopy and PCR7 to the guinea pig model.

With such advancement, it is inevitable to uncover certain parameters in these models that may be complicating assessment of efficacy of new candidate vaccines. These complications may not be directly implying that our choice and hence “pipe-lining” of new vaccines is erroneous, but certainly imply that in the real world matters may be far more complex. Four possible complications, some of which may be important and some more trivial, are discussed in this review.

Induction of Foxp3+ T cells

Regulatory T cells includes both CD4 and CD8 that can inhibit the activity of other effector T cell populations8,9. Apparently, there are multiple subsets of these cells, but most of them seem to fall into the phenotype CD25hi and Foxp3+ (with the caveat that Foxp3 is currently regarded as the most useful marker, but there appear to be minor subsets that are Foxp3-negative), of which some secrete certain cytokines, such as IL-10 and TGFβ.

In the mouse model, most studies have only demonstrated a small generation of such cells to H37Rv strain10,11, but much more virulent clinical isolates can potently generate this subset. In a recent study12, the highly virulent strain HN878 has been shown to potently generate CD4 cells making IFNγ, but this response decays rapidly and is replaced by a substantial influx into the lungs of CD4 cells.
expressing CD25hi Foxp3+, many of which are also positive for IL-10 (Fig. 1).

Can the propensity of such virulent clinical isolates to induce regulatory T cells confound expression of vaccine induced protection? A recent study in the mouse suggests that it does not interfere with BCG-induced protection in the short-term model, but this has not been tested as yet in the guinea pig model which unlike the mouse develops substantial inflammation and necrosis (even eventually in BCG vaccinated animals), elements of pathology that regulatory T cells appear to be generated against to limit lung damage.

**Induction of memory T cell subsets**

Studies in our laboratory that showed that memory immunity (induced by clearance of the infection by drug treatment) can be transferred by CD4+ spleen cells, were performed in a simpler era when memory was thought to be mediated by a single discrete T cell population. Two decades later it is now apparent that there are at least two major memory T cell subsets, represented within both the CD4 and CD8 subsets. The two memory populations differ in location, speed of recall, patterns of cytokine secretion, and phenotypically in terms of expression of CD62L and chemokine receptor CCR7. Hence, cells that are CD62Lhi and CCR7+ are found in lymphoid tissues and are called central memory cells (T CM), whereas CD62Llo CCR7-negative effector memory cells (T EM) are found in non-lymphoid tissues including lungs. The general concept is that T EM represent an initial responding population located in the periphery, while T CM represent a highly-reactive powerful subset providing a second level of protection at the lymphoid tissue level when the infection should reach that deep into the body.

The majority of information on these subsets has been gained from viral infection models, in which the immune system is usually capable of completely
clearing the infection. Under these conditions there is
the establishment of a substantial $T_{CM}$ response. In
studies in our laboratory, we have found that both
BCG vaccination (not challenged) and alternatively
the establishment of chronic $M. tuberculosis$, induce a
substantial $T_{EM}$ influx into lungs, which is stable over
a long period of time. In contrast, far lower numbers
of $T_{CM}$ can only be found in the splenic lymphoid
tissue, with extremely few in the lungs even in the
case of chronic infection (Fig. 2). We can only
speculate as yet, but this may imply that BCG is not
good at inducing $T_{CM}$. Even if $T_{CM}$ are generated by
chronic $M. tuberculosis$ infection, they are being
continuously driven into effector populations (bearing
in mind it is currently difficult to distinguish effector
$T$ cells and $T_{EM}$ based on cell surface phenotype
alone).

This has several implications, the biggest of which
is that BCG is a relatively poor vaccine, the efficacy
of which wanes as children grow older, because it is a
poor inducer of $T_{CM}$? This may also explain why
prime boost regimens appear to be superior, in that
they are potentially expanding the $T_{CM}$ subset (that
can be tested).

**Location of extracellular organisms in lung necrosis**

This may be trivial in the case of vaccine efficacy,
but the reader may appreciate that it may be
fundamental to successful drug treatment. Guinea pigs
develop a heterogeneous pathology profile in lungs
with necrotizing primary lesions and solid post-
primary lesions established by bacterial dissemination
through blood and local lymphatics. After initial
chemotherapy, we have shown that secondary solid
lesions resolve, but primary lesions remain. Initiation
of partial dystrophic calcification leaves an acellular
“rim” on perimeter of the central necrosis, and within
this structure that bacteria survive after initial drug
therapy (Fig. 3). We now have evidence that these
bacilli are extracellular, and appear to be in some sort
of biofilm which includes host actin and DNA, and
free mycolic acids exuded by the bacterium. This may
be forming some sort of shell around the bacilli, and
may explain their resistance to drugs.

If a vaccine, like BCG, prevents the lung necrosis,
then this observation is probably not an issue. But if
the vaccine works more slowly or has a limited period
of protection (perhaps related to memory subsets as

![Graphs showing CD4 $T_{EM}$ and CD4 $T_{CM}$ in lung and spleen](image-url)
described above) and some degree of necrosis gets established (an event that eventually occurs even in BCG-vaccinated guinea pigs) then a subset of bacteria that is extracellular will become established. These would be resistant to chemotherapy and unrecognizable by the TH1 response, since they are no longer inside macrophages, and hence, could pose a serious intractable problem.

Protection against isolates of high/extreme immunopathology

While a few clinical isolates of *M. tuberculosis* have been studied, this has mostly been performed in non-necrotic mouse model. We have recently collected a series of clinical isolates with the criteria- (i) they are associated with an actual outbreak in USA; (ii) they represent both W-Beijing and non-Beijing strains, and (iii) some were MDR isolates. These are described in details elsewhere [Palanisamy et al., *Tuberculosis*, In Press].

What was unanticipated in our study was the extreme range of lung pathology we subsequently observed in the guinea pig model. While some gave pathology similar to usual laboratory strain H37Rv, this was very much the bottom end of the range, with certain strains giving extremely severe lung damage and lymph node cavitation (Fig. 4). BCG can prevent necrosis induced by H37Rv, but can it do anything to stop these “real” strains. In fact would any of the new candidates currently in the pipeline be able to do so?

References


Fig. 3—A primary lesion in a guinea pig infected with *M. tuberculosis*. The lesion has mostly mineralized, but there is a residual “rim” of acellular debris/necrosis in which drug-tolerant bacilli can persist.

Fig. 4—Three W-Beijing strains show a substantial range of virulence and pathology in the guinea pig model. H37Rv, against which most vaccines are tested, has pathology similar to TN7642.


