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Bacterial host resistance models in the evaluation of immunotoxicity

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Abstract

To assess potential immunomodulatory effects of a drug, pollutant, or natural product, an analysis of an exposed host's ability to resist challenge with a viable bacteria is one of the best gauges. Many factors govern whether a host exposed to a test agent and then infected becomes ill or dies at rates greater than infected control counterparts. Beyond the status of the host's immunocompetence, a bacterium's route of entry into the host and its inherent virulence are important variables determining how (and rate at which) an infection resolves. A pre-determination of endpoint(s) to be defined is critical during planning of resistance assays. If a study is to determine overall changes in immunocompetence due to exposure (regardless of regimen or dosage of test agent), then assessing shifts in morbidity/mortality at a defined lethal dose [LD_x] value for the chosen route of infection would suffice. However, if a study is to define extent of immunomodulation in a particular body organ/cavity—or specific alterations in particular aspects of the humoral or cell-mediated immune responses—then careful selection of the pathogen, dose of the inoculum, means of infection of target site, and extent of the post-infection period to be examined, need to be made prior to host exposure to the test toxicant. This review will provide the Reader with background information about bacterial infections and how endpoint selection could be approached when designing resistance assays. An overview of protocols involved in the assays (e.g., bacterial preparation, host infection, post-infection endpoint analyses) and information about three bacteria that are among the most commonly employed in resistance assays is provided as well.

Keywords: Bacteria host resistance; *Listeria monocytogenes*; *Klebsiella pneumoniae*; *Streptococcus pneumoniae*; Vanadium; Ozone; Pulmonary clearance

1. Introduction

Among the various protocols suggested in the Tier I and Tier II approaches to assessing potential immunomodulatory effects of any given drug, pollutant, or natural product [1], an analysis of the ability of the exposed host to resist challenge with a viable bacteria remains one of the best gauges of overall alteration in the host's immune system.

Exposure to a live bacterial organism is probably the best reflection of a real-world immune challenge scenario for any host. In fact, humans and animals are continually exposed to a variety of infectious agents. However, even after infection, most hosts do not necessarily go on to develop clinically defined disease states. Clearly, there are many factors that govern whether the infected host eventually becomes ill. Beyond the status of the host's immunocompetence, the route of entry into the body by the bacterium and more critically, its inherent virulence, are important variables in how the infection might resolve.

In the realm of immunotoxicology, the application of host resistance assays to assess immunomodulatory potentials of any test agent require that the investigator first determine which endpoint(s) they seek to define. For example, if the study is meant to determine overall changes in immunocompetence due to exposure (regardless of the precise exposure regimen or dosage(s) of the test agent used), then an assay to assess morbidity/mortality using previously defined lethal dose values (for each given route of infection; LD_x) for any particular bacterium could suffice. In contrast, if the investigator seeks to define the extent of any induced immunomodulation within a particular body organ or cavity (i.e., lungs, liver, peritoneal cavity)—or specific alterations in particular aspects of either the humoral or cell-mediated arms of the immune response—then a much more careful selection of

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the pathogen (including accounting for virulence or factors influencing same), the dose of the inoculum, the means of infection of the target site, and the extent of the post-infection period to be examined (and timepoints for analyses of bacterial burdens, etc. therein), needs to be made prior to the exposure to the test toxicant.

2. Bacterial infections—overview

Choosing a particular bacterium for infecting an animal model to assess the immunomodulatory potential of a test agent should optimally reflect what could occur in an agent-exposed human [2]. The infection model should be one that simulates a common/prevalent human disease and whose disease course, pathogeneses, and ultimately, immunologic resolution correspond to what occurs in a human host. Furthermore, the means of infection should both follow the natural route for the pathogen and the dose be substantially low so as to mimic what would naturally occur in man (and also so as not to overwhelm the animal's immune system from the start). As noted in the cited Bradley review, "all of these criteria can be achieved only rarely, but a model should satisfy as many of these conditions as possible".

In general, infections caused by bacteria are deemed either acute (purulent), chronic (granulomatous), or toxigenic. Those falling into the first category are of a short duration and the acute course of the immune response in characterized by leukocyte accumulation at the infection site (and concomitant development of local purulence). In these types of responses, the uptake and intraphagolysosomal killing of the bacteria (with initial phagocytic uptake aided by a presence of opsonins) leads to quick resolution of the infection. The bacteria that lead to this type of infection are generally termed as extracellular bacteria. The great majority of cocci and most Gram-negative rods fall into this category (Table 1).

Those bacteria that give rise to chronic, granulomatous type of infections also undergo ingestion by leukocytes; however, rather than all being killed thereafter, some survive and are able to proliferate within the cells. Ultimately, removal of these facultative intracellular pathogens requires enlistment of T-lymphocytes to both enhance the recruitment and assembly of monocytes (leading to the formation of local granulomatous lesions) and to maximize the bactericidal activity of the infected and uninfected cells. This lesion formation is critical in that within it, the cells are tightly packed to maximize cell-to-cell interactions and for the effective containment and destruction of the facultative organism (as well as mitigation of danger of further bacterial invasion or spread of infection). Organisms that fall into this classification are commonly members of the species *Mycobacterium*, *Brucella*, and *Yersinia*, as well as those of *Listeria*, *Legionella*, and two types of *Salmonella* (e.g., *S. typhi* and *S. paratyphi*) and *Pseudomonas* (e.g., *P. mallei* and *P. psudomallei*) [3].

The final class of infections, i.e., toxigenic, are the type that are the result of the production of bacterial toxins. These fall into two categories—the exotoxins (released by the viable bacteria into the host tissues) and the endotoxins (released primarily as a result of damage to the bacteria itself). In all instances, the formation and release of specific antitoxin by the host are required for neutralization of these poisons and ultimate resolution of the infection. Exotoxins normally display strong specificities for targeted tissues; toxins from *Clostridium tetani* and *Clostridium botulinum* specifically affect spinal neurons and neurosynapses, respectively, while those from *Vibrio cholera* and *Vibrio pertussin* act on gut columnal epithelia and respiratory epithelia (primarily the ciliated types), respectively. In contrast, endotoxins affect multiple cell types, tissues and organs. In either case, the potential release of these products needs to be taken into account by the Investigator during the design of resistance studies; variations in the presence of these agents will contribute a confounding factor (beyond any changes in integrity of the host immune system) that needs to be analyzed during determination of mechanisms by which the test agent caused a change in overall host resistance.

Table 1
Common facultative intracellular and extracellular bacteria

Facultative intracellular bacteria
Listeria monocytogenes
Mycobacterium tuberculosis
Mycobacterium leprae
Salmonella typhi
Legionella pneumophila
Brucella spp.
Yersinia spp.
Extracellular bacteria Gram-negative rods
Bacteroides fragiles
Escherichia coli
Haemophilus influenzae
Klebsiella spp.
Enterobacter spp.
Proteus spp.
Pseudomonas spp.
Salmonella (S. typhi and paratyphi) spp.
Gram-negative cocci
Neisseria
Streptococci
Staphylococci
Pneumococci

3. Defining host resistance endpoints to be examined

Clearly, the ultimate goal of any immunotoxicological investigation is to define whether or not a test agent adversely impacts upon the host immune system. In the classic sense when employing bacterial resistance assays, an outcome is deemed adverse when at some dose, the agent causes an increased susceptibility/severity in the bacteria-specific induced disease as compared to values seen in control counter-parts.

At the most basic level, experimental resistance to any organism can be assessed generically via analyses of mortality/survival (a "frank test"; [4]). Studies of this type pro-