Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection 7 (2005) 1263–1275
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

A model of immunity to *Burkholderia pseudomallei*: unique responses following immunization and acute lethal infection

Glen C. Ulett [a,b,*], Justin T. Labrooy [b,c], Bart J. Currie [d], Jodie L. Barnes [e], Natkunam Ketheesan [b,e]

[a] *Department of Infectious Diseases, St. Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, TN 38105-2794, USA*
[b] *School of Medicine, James Cook University, Townsville, Qld. 4814, Australia*
[c] *Internal Medicine Service, Royal Adelaide Hospital, Adelaide, SA 5000, Australia*
[d] *Menzies School of Health Research, Charles Darwin University and Northern Territory Clinical School, Flinders University, Royal Darwin Hospital, PO Box 41096, Casuarina, NT 0811, Australia*
[e] *Department of Microbiology and Immunology, School of Biomedical Sciences, James Cook University, Townsville, Qld. 4811, Australia*

Received 13 April 2004; accepted 18 April 2005

Available online 08 June 2005

Abstract

Burkholderia pseudomallei, the etiological agent of melioidosis, causes significant mortality in endemic regions, but little is known regarding the immune mechanisms required for successful protective immunity. To establish a model of immunization that could be used to study this we screened a library of *B. pseudomallei* strains for immunogenicity in mice. BALB/c mice were immunized with test strains, and 2 weeks later were given a lethal challenge (LC) of virulent *B. pseudomallei*. Among 49 strains tested, a single strain, CL04, exhibited strong immunoprotective capacity. Interestingly, CL04 had been cultured from a patient with chronic colonization of *B. pseudomallei*, which is a rare phenomenon. Mice immunized with $0.1 \times LD_{50}$ (5×10^3 CFU) of CL04 had significantly better survival and lower bacterial loads after LC compared to naïve controls. Dose–response analysis demonstrated more robust immunity after higher immunizing doses, and bacterial inactivation by gamma irradiation diminished the protective effect, indicating a requirement for viable organism for immunity. CL04-induced immunity was demonstrated both in *B. pseudomallei*-susceptible BALB/c and -resistant C57BL/6 mice. We investigated the gene profile of CL04-induced immunity by analyzing responses to immunization using cDNA microarray. Unique responses involving granulocyte macrophage colony stimulating factor (GM-CSF), the proapoptotic regulator Bad and cyclin-dependent kinase (CDK5) were detected in immunized mice, but these responses were absent in naïve-LC mice. Further, responses differed between mouse strains, indicating dependence on host genetic background. This model will be useful in identifying elements of the immune response required for successful adaptive immunity against *B. pseudomallei*.

Keywords: Burkholderia pseudomallei; Melioidosis; Immunization

1. Introduction

Burkholderia pseudomallei is a select agent for which there is no vaccine currently available [1]. The organism causes melioidosis, a disease with multifarious clinical manifestations and has been listed as a potential biowarfare agent. In endemic regions such as Northern Australia and Thailand, *B. pseudomallei* exists in the environment as a saprophyte [2]. Infection is acquired through skin abrasions or inhalation of contaminated soil or surface water. Clinical disease presents along a spectrum of severity ranging from acute fulminating sepsis, which carries high mortality rates to chronic persistent infection that is difficult to resolve with current antibiotic therapies. Immunocompromised individuals including people with diabetes are particularly susceptible to infection [3]. The acute and chronic forms of human melioidosis have

Abbreviations: CDK5, cyclin-dependent kinase; GM-CSF, granulocyte macrophage colony stimulating factor; IHA, indirect hemagglutination; LC, lethal challenge.

* Corresponding author. University of Queensland, School of Molecular and Microbial Sciences, St. Lucia, Brisbane, Queensland, Australia 4072. Tel.: +6107 3365 3830; Fax: 61 07 3365 4699.
E-mail address: g.ulette@ug.edu.au (G.C. Ulett).

1286-4579/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2005.04.013

been successfully modeled in mice, where the BALB/c strain is acutely susceptible to infection compared with C57BL/6 mice, which are relatively resistant [4]. In patients, relapse of disease is not uncommon [5] illustrating poor efficacy of current therapies and an intrinsic ability of *B. pseudomallei* to evade antibiotics and the host immune response.

Immunity to *B. pseudomallei* has been difficult to study because of the highly variable nature of the disease and broad differences in virulence of individual *B. pseudomallei* strains [6]. Hence, little is known about what comprises successful adaptive immune responses to *B. pseudomallei*. Establishment of a mouse model of *B. pseudomallei* infection has contributed to our understanding of the pathogenesis of primary infection [4]. In primary infection, cytokine responses involve the proinflammatory regulators TNF-α and IL-6 rather than polarized T Helper patterns [7]. These observations of primary immune responses in mice correlate well with data derived from patient studies [8]. Functional studies have also shown that IFN-γ is required for host survival following primary infection in mice [9]. A well-characterized model of experimental immunization leading to protection against secondary lethal infection, which is required to study adaptive immune responses to *B. pseudomallei* has not been previously established. In a report by Warawa and Woods (2002), it was noted that a vaccine candidate developed based on capsule-O-PS (LPS)-flagellin conjugates was not tested effectively due to the lack of a suitable animal model of *B. pseudomallei* infection [1].

To address the issue of adaptive immunity to *B. pseudomallei* infection, we undertook the current study to establish and characterize a model of *B. pseudomallei* immunization. We screened a library of *B. pseudomallei* strains for immunogenicity in mice and having identified the most immunogenic strain, determined parameters of immunization required for immunity. The host response to immunization was investigated by gene microarray. A single *B. pseudomallei* strain was identified with strong immunoprotective capacity, and comparison of the immune response induced by immunization and acute infection revealed unique responses in each case. Finally, these unique immunization-associated responses were shown to be dependent on host genetic background.

2. Materials and methods

2.1. Bacterial strains

Details of bacterial strains are given in Table 1 and additional details are available elsewhere [6]. These test strains were chosen because they represent a diverse cross-section of clinical and environmental isolates, which have been characterized previously. *Burkholderia thailandensis* strains were originally described by Wuthiekanun et al. [10] and were obtained from Dr. Tim Inglis [11] for this study. Virulence of strains not previously characterized was determined by LD_{50}

Table 1
B. pseudomallei test strains and doses used for immunization of BALB/c mice against lethal *B. pseudomallei* infection

Strain[a]	Immunization Dose (CFU)	*x*[b]
Naïve	PBS-LC	1
Non-LC	PBS-PBS	2
ATCC 23343	3.40×10^5	3
CL01	4.00×10^5	4
CL02	1.30×10^5	5
CL03	1.00×10^5	6
CL04	8.50×10^3	7
CL05	3.50×10^3	8
CL06	1.60×10^3	9
CL07	2.00×10^3	10
CL08	2.00×10^3	11
CL09	2.00×10^3	12
CL10	2.50×10^3	13
CL11	8.00×10^2	14
CL12	4.20×10^2	15
CL13	3.00×10^2	16
CL14	2.40×10^2	17
CL15	2.10×10^2	18
CL16	2.00×10^2	19
CL17	1.60×10^2	20
CL18	1.70×10^2	21
CL19	1.00×10^1	22
CL20	Lethal	–
CL21	Lethal	–
CL22	Lethal	–
CL23	Lethal	–
CL24	Lethal	–
CL25	Lethal	–
CL26	Lethal	–
VE01	1.00×10^4	23
VE02	1.00×10^4	24
VE03	1.00×10^3	25
VE04	2.50×10^2	26
VE05	Lethal	–
VE06	Lethal	–
EN01	1.75×10^6	27
EN02	1.30×10^4	28
EN03	1.90×10^4	29
EN04	2.00×10^3	30
EN05	1.36×10^3	31
EN06	2.00×10^3	32
EN07	5.00×10^2	33
EN08	4.80×10^1	34
EN09	4.00×10^2	35
EN10	Lethal	36
EN11	Lethal	37
AR01	5.00×10^5	38
AR02	1.00×10^6	39
AR03	5.50×10^5	40
AR04	3.00×10^6	41
AR05	2.00×10^7	42

[a] Naive, control mice that received only PBS as mock-immunization prior to LC; non-LC, control mice that received only PBS, followed by PBS; CL, clinical strain; VE, veterinary strain; EN, environmental strain; AR, arabinose assimilating environmental isolate; details of strains used for immunization available in [6], details of AR strains available in [11]. CL07 = NCTC 13179 and CL24 = NCTC 13178. Immunizing strains with $LD_{50} < 50$ CFU (CL20-26, VE05-06) were consistently lethal within 72 h of immunization and were not tested.

[b] Designated number of immunizing test strain, shown on *x* axis in Fig. 1.