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Introduction

The use of animals as models for microbiological infections is a fundamental part of infectious disease research. The intent for the use of animals as models of disease is to establish an infection that mimics that seen in humans (Druilhe et al., 2002). The goal is to seek how the infection develops, and by what the infection can be thwarted. Hence, animal models can be proposed to study the virulence and pathogenicity of a microorganism or to screen candidate antibiotics for their performance in eliminating the infection. Because *Acinetobacter* frequently develops resistance to antibiotics, experimental models of infection are useful in pharmacokinetic assays and/or for the study of various antibiotic regimens. Study on virulence is still in an elementary stage.

The use of animal models must be approved by the Ethical Committee for animal experiments.

Importance of Various Parameters in Experimental Models

The objective of an infections model is to mimic human infection in order to obtain relevant therapeutic implications. Experimental models make possible the study of in-vivo conditions and approach the complex interaction that exists between the antibiotic, the bacterium and the host. The low pathogenicity of *Acinetobacter baumannii* has been widely discussed. The lethal dose of 50% is about 10^8 in an immunocompetent mouse. The main difficulty in experimental model due to a non pathogenic strain is to trigger off the infectious process. In the various experimental models discussed in this chapter, different methods have been used such as the immunosuppression model of either long or short duration, and the use of porcine mucin mixed with bacterial inoculum. To be relevant, an experimental model of infection must be appropriate to the type of

M.L. Joly-Guillou (Angers), M. Wolff
Service de Réanimation Médicale, CHU Bichat-C Bernard, 75018 – PARIS, France

infection (Wiles et al., 2006). For example, in pneumonia models, infection must be compartmentalized such as proved by a significant difference between the quantitative bacterial count in lung and in blood. A model of infection showing the same inoculum value in the blood and at the site of infection will represent a sepsis model with a weak relevance to the organ infection. Overall, the experiment must be reproducible under strictly identical conditions. The inoculum must be adapted to the objectives of the model (acute or chronic infection) (Joly-Guillou et al., 1999). Finally, to be relevant in humans, administration of drugs in animals must take into account the pharmacokinetic/pharmacodynamic specificity of the drug in humans.

Mouse Models of Pneumoniae

The selection of an animal depends on the infection and the focus of the study. The physiological reaction to an infection and the nature of infection must mirror as closely as possible the situation in humans. Experimental models of acute systemic infection and urinary tract infection due to *Acinetobacter* spp. were first developed in mice in 1985 to examine some virulence factors, and the efficacy of therapy with tetracycline and aminoglycosides or sulbactam (Obana et al., 1985; Obana, 1986). An inoculum of at least 10^6 cfu was required to kill a mouse, but LD_{50} values were considerably lower in the presence of hog gastric mucin (10^3–10^4 cfu/mouse). It was shown that mixed infection of *Acinetobacter* resulted in a greater virulence than infection with one pathogen, and that the slime of *Acinetobacter* enhanced the virulence of *E. coli*, *S. marcescens*, or *Pseudomonas aeruginosa*. Since pneumonia is the most serious nosocomial infection due to multiresistant *Acinetobacter*, effective mouse models of pneumonia have been performed (Table 1). Generally, mice were anesthetized by IP injection of sodium thiopental (Rodriguez-Hernandez et al., 2000) or ketamine (Bernabeu-Wittel et al., 2005), sodium pentobarbital (Joly-Guillou et al., 1997), or by inhalation of isoflumane (Knapp et al., 2006). Bacterial inoculation was performed by trachea cannulation with a blunt tipped needle or intranasally.

A first model of acute pneumonia due to *A. baumannii* was developed in 1997 (Joly-Guillou et al., 1997) leading to death of 85% of the animals within 3 days. Since a significant difference was found in susceptibility to *Acinetobacter* between various mouse strains, the infection model was developed in 6-week-old, specific pathogen free, C3H/HeN mice (Table 2). Animals were rendered transiently neutropenic in order to favor the onset of infection. Cyclophosphamide was given intraperitoneally (ip) (150 mg/kg of body weight), 4 and 3 days before inoculation and neutropenia was observed at day 0 and 1. A transient leukocytosis (12,000/mm3) was observed on day 3. Mice were inoculated by trachea-cannulation, with 50µl of an inoculum of 10^8 cfu/ml. The majority of deaths occurred between day 2 and day 3 (Table 3), when histopathological

Table 1 Experimental models of *Acinetobacter pneumonia*

	Joly-Guillou et al. (1997)	Rodriguez-Hernandez et al. (2000)	Knapp et al. (2006)	Bernabeu-Wittel et al. (2005)
Animals	C3H/HeN Mice 6 week old	C57BL/6 N Mice 8–10 week old	C57BL/6 Mice 8–10 week old	Dunkin-Harthley Guinea-Pig
Immunosuppression	Transienlly J0 + J1	Immunocompetent	Immuno-competent	Immuno-competent
Inoculum	50 µl (10^8cfu/ml)	50 µl (10^8cfu/ml) + 30 µl Mucine Porcine	10^7 cfu/ml	10^9 cfu/ml +Mucine Porcine
Inoculation	Intra-tracheal	Intra-tracheal	Intra-nasally	Trans-tracheal After surgical exposure
Markers	Mortality rate (85%)	Mortality rate (100%)	Cytokine/Chemokine	Mortality rate (0–10%)
	Mean day of death (J1–J2)	Mean day of death (J2–J3)	PMNs Lymphocytes Alveolar macrophages	–
	Mean bacterial count/s of lung 10^9 cfu/gr	Mean bacterial count/s of lung 10^{10} cfu/gr	Mean bacterial count/s of lung 10^6 cfu/grl	Mean bacterial count/s of lung 10^8 cfu/gr
	Mean bacterial count/ml of blood 10^3/ml	–	–	–
	Histologic examination in lung	Histologic examination in lung	Histologic examination in lung	Histologic examination in lung
Objectives	PK-PD Treatment	PK-PD Treatment	Host response	PK-PD Treatment

Table 2 Comparison of susceptibilities of three strains of non neutropenic mice to *A. baumannii* 48 hours after intratracheal inoculation of 5×10^6 CFU

Mouse strains (Mean wt [g] ± SD)	No. of deaths/no. of mice tested	Mean maximum wt loss (g) ± SD (%)	Mean $\log_{10}$ CFU/g of lung ± SD
Swiss Webster (26.28 ± 1.5)	3/15[a]	2.74 ± 2 (10.42)[a]	8.73 ± 0.68
C3H/HeN (19.5 ± 0.96)	9/15	4.19 ± 1 (21.4)	9.1 ± 0.8
C57BL/6 N (16.44 ± 0.5)	10/14	3.23 ± 1.1 (19.6)	9.26 ± 0.3

[a] $P = 0.02$ versus the other strains of mice (Joly-Guillou et al., 1997).

examination showed abscess formation, with extensive infiltration of polymorphonuclear leukocytes and exudate in alveolar spaces and bronchial lumens. Pneumonia is the primary focus of infection: although the majority of animals were bacteremic, quantitative blood culture yielded relatively low bacterial counts compared with those in lungs. This model was suitable for pharmacokinetic studies and for studies of efficacy of antibiotic therapy. It should be suitable for virulence studies, but experiments are reproducible for one *A. baumannii* strain only, whereas various strains have various virulence effects related to their phenotype and genotype characteristics (personal data).

A pneumonia model was developed by Rodriguez-Hernandez et al. (2000) in immunocompetent pathogen-free C57BL/6 N female, in order to evaluate the efficacy of antibiotic regimens. To favor the onset of the infectious process, inoculum was mixed to porcine mucin. Although the mice were bacteremic, bacterial counts were performed in lungs, and not in blood. Non treated mice died in the first 48 hours, and a pattern of acute inflammation consistent with pneumonia was observed.

The objectives of these models were to evaluate the efficacy of antibiotic regimens. The global synthesis of the results showed that sulbactam was as efficacious as imipenem in term of survival, sterility of lung, and bacterial clearance from lungs and blood (Rodriguez-Hernandez et al., 2001). In

Table 3 Characteristics and duration of *A. baumannii* lung infection in C3H/HeN mice

Post infection day	No. of neutrophils mm^3	Mean body wt (g) ± SD	Cumulative mortality(%)[a]	Mean CFU/g of lung ± SD
0	300	19.3 ± 1.06	0	7.3 ± 0.2
1	220	17.7 ± 1.2	8	9.0 ± 0.9
2	4,120	16.7 ± 1.4	45	9.4 ± 0.8
3	12,000	17.3 ± 1.6	81	8.6 ± 1.2
4	4,000	17.8 ± 1.5	85	7.7 ± 1.4
7	5,200	19.2 ± 1.1	85	$< 5 \times 10^2$

[a] Seventy-four mice were tested (Joly-Guillou et al., 1997).

infections due to moderately susceptible strains to imipenem, this drug can still be used combined with another active drug such as rifampin, sulbctam, doxyycline, or lévofloxacine (Rodriguez-Hernandez et al., 2000). Rifampin alone presents an excellent bactericidal effect on susceptible *Acinetobacter*, but it must not be proposed alone in *Acinetobacter* infections (Pachon-Ibanez et al., 2006), unlike levofloxacine (Joly-Guillou et al., 2000). The combination of aminoglycosides such as amikacin or tobramycin with sulbactam, doxycycline, or rifampin when active, could be proposed as an alternative therapy in *Acinetobacter* infections (Bernabeu-Wittel et al., 2005; Rodriguez-Hernandez et al., 2000; Montero et al., 2004). Nonclassical combinations of ticarcillin plus clavulanate with sulbactam or rifampin should be considered as an alternative (Wolff et al., 1999). The efficacy of colistin in pneumonia infection remains discussed, even if in vitro studies using MICs have suggested that this is the most active alternative. In some cases of pan-resistant *A. baumannii*, colistin could be the only drug available for treatment (Montero et al., 2002, 2004).

To study the host-pathogen relation, another type of acute *A. baumannii* pneumonia model, which allows the in vivo investigation of molecular mechanisms of the host defense during *Acinetobacter* infection, has been developed (Table 1). This model has been useful for the studies of the innate and adaptive immune responses to *A. baumannii* (Knapp, 2006; Renckens, 2006; Erridge et al., 2007).

Guinea-Pig Model of Pneumonia

A guinea-pig pneumonia model was developed to assess the efficacy of antibiotic regimens, pharmacokinetics, and pharmacodynamic parameters (Bernabeu-Wittel et al., 2005). An inoculum of 10^9 cfu/ml mixed with porcine mucin solution was introduced slowly by transtracheal inoculation after surgical exposure of the cervical trachea. This model of acute pneumonia presented a low mortality rate in immunocompetent animals with a bilateral pneumonia and high bacterial concentrations in the lungs of animals. From these experiments, the authors concluded that combined use of imipenem and amikacin was less efficient than monotherapy, probably because of a drug-drug interaction that resulted in decrease pharmacokinetic and pharmacodynamic parameters for both antimicrobial agents.

Rabbit Models of Endocarditis

For studies of the efficacy of Sulbactam and Colistin, Rodriguez-Hernandez et al. (2001, 2004) describe a New-Zealand immunocompetent rabbit model of experimental endocarditis. Four days after insertion of an intracardiac catheter

in the left ventricle, the rabbits were inoculated with a suspension of 10^8 cfu/ml through the marginal vein of the ear. Blood samples taken 24 days after inoculation confirm the onset of endocarditis. The authors showed that colistin was effective in the clearance of *A. baumannii* bacteremia caused by susceptible strain, but may not be a suitable treatment for endocarditis. In this model, imipenem was more efficacious than sulbactam in bacterial clearance from vegetation caused by susceptible strains, reflecting the higher and persistent time above MICs of imipenem ($t >$ MIC) compared with sulbactam (2.12 hours versus 1.17 hours).

Rat Model of *Acinetobacter* Thigh Infection (Pantopoulou, 2007)

A model of thigh infection has been developed in neutropenic rat, in attempting to simulate clinical practice and to measure the effect of Colistin on bacterial eradication, survival and, its interaction with co administered rifampin. After induction of neutropenia, rats were challenged by the intramuscular injection of 1 ml of inoculum in the left thigh under slight anesthesia. The right jugular vein was recognized by midline neck incision and catheterized by a 26 G catheter to be used for drug infusion. Colistin was effective in prolonging survival. Its activity was enhanced after co-administration with rifampin.

Mouse Model of Gastritis (Zavros et al., 2002; Rathinavelu et al., 2003)

Acinetobacter lwoffii was used to colonize the stomach of C57Bl/6 mice in comparison with *Helicobacter pylori*. Mice were pretreated by orally intubating them with streptomycin (5 mg/ml) for 3 consecutive days. After 48 hours, mice were orally inoculated with a catheter, three times over a period of 3 days, with 10^8 organisms (per 200 μl). Mice were sacrificed at 2, 3, and 4 months after oral inoculation.

Acinetobacter lwoffii infection in mice was similar to that observed with *Helicobacter* infections. *Acinetobacter* can trigger gastritis by the way of virulence factors. For example, the urease activity favors the colonization of the mouse stomach. The fimbriae help to adhere to human gastric epithelial cells via *A. baumannii*. The effects observed in the mouse were similar to those observed with *Helicobacter pylori* such as hypergastrinemia and stimulation of cytokine release. These results could have clinical relevance in patients with low gastric acid secretion due to disease or acid-suppressing drugs.

Experimental Infection of Body Lice (Houhamdi and Raoult, 2006)

Previous studies have reported the isolation of *A. baumannii* from body lice of homeless patients. To study how the body louse acquired *Acinetobacter*, a rabbit was infected by infusing 2×10^6 cfu of the louse strain of *Acinetobacter*. Body lice that fed on rabbits infused with A*cinetobacter* species demonstrated a generalized infection, but the body lice did not transmit their infection to the nurse rabbit by bite while feeding or to their progeny (eggs and larvae). Only *A. baumannii* was pathogenic for the body louse.

Conclusion

Models occupy an essential position in the study of infectious disease. Deliberately induced infections in well-defined animal models provide much useful information about disease processes in an approximation of their natural context. Despite this, animal models are not the natural disease process and the results of experimental infections require careful interpretation and any extrapolation to humans should be made with great cautions.

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