Invasive Aspergillosis Animal Models (IAAM): Standardized Murine and Guinea Pig Models of Invasive Pulmonary Aspergillosis

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ABSTRACT

Background: Invasive pulmonary aspergillosis (IPA) is clinically relevant with limited treatment and diagnostic options. Evaluations of diagnostic methodologies and therapeutic options against IPA have been performed in a wide variety of animal models. Recently, extensive investigations into the *Aspergillus* genome have received great attention; however, animal models have only been used sparingly for genomic evaluation of this disease organism. The variations of animal models and their design make comparison of in vivo studies difficult. Thus, we have addressed this critical need to develop uniform standard model systems to evaluate new diagnostic targets and methodologies. **Methods:** A recently awarded contract from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (N01-AI-30041), supports the development of standardized animal models of invasive aspergillosis (IA) to evaluate new diagnostic targets and tools to answer key questions in this disease. The Invasive Aspergillosis Animal Models (IAAM) study group is designed to be a resource to provide training and experimental studies for the *Aspergillus* research community at large. **Results:** We have developed both mouse and guinea pig models of IPA using two different types of inhalation chambers. Both models present the key features of reproducible pulmonary infection using either a low-cost acrylic chamber (Sheppard, AAC 2004) or a large-scale Madison aerosol chamber, which allows simultaneous challenge of up to 126 mice or 18 guinea pigs, predictable mortality, ease of duplication, and they recapitulate human disease. Simple serial blood sampling is a feature of the guinea pig model that enhances monitoring of surrogate markers of infection. These models serve to encourage the development of diagnostic methodologies and tools against IPA. To date, 15 investigators from academia and industry have initiated collaborative efforts under this contract to study the effects of gene expression or gene deletions, diagnostic methods and vaccines, as they relate to development of new diagnostic targets. Finally, the contract provides for the creation and maintenance of an *Aspergillus* database, whereby qualified investigators may share details of their strains and experiments. **Conclusions:** This contract, through these key features, provides the *Aspergillus* research community with the mechanism for evaluation of genomic targets using standardized animal models of IPA in order to improve the diagnosis and therapy of IA. These studies are ultimately aimed at improving patient care and reducing mortality in this often fatal disease.

INTRODUCTION

Animal models have been utilized to evaluate both the diagnosis and treatment of IA and have also been used to assess pathogenic and virulence features of the organism [1,2]. However, substantive efforts to assess virulence and pathogenicity and to investigate or identify new diagnostic targets in *Aspergillus* have been hampered by the lack of a standardized animal model of IA [1,3]. Extensive efforts are underway in *Aspergillus* that will significantly expand the genomic information regarding *Aspergillus*, so that the potential for identifying new diagnostic and therapeutic targets which are critically needed for this often lethal infection is great. One approach to providing standardization to *Aspergillus* animal models has been the award of a contract by the National Institutes of Health/National Institute of Allergy and Infectious Disease (NIH/NIAID) to establish and standardize animal models of IA including murine and larger animal species with both pulmonary and disseminated infection [1,3,4]. Within this framework, our goal is to provide the basis for evaluating genomic targets to improve the diagnosis and treatment of IA, ultimately resulting in improved outcomes of patients with this frequently fatal infection.

MATERIALS and METHODS

IAAM contract: Because of the critical need identified for development of new diagnostic markers in invasive aspergillosis as well as the refinement and better understanding of currently available methods, the National Institutes of Health/National Institute of Allergy and Infectious Diseases recently awarded an contract (NIH-NIAID-N01-AI-30041) to establish standardized animal models of invasive aspergillosis to address the critical needs in this field and to further advancement of diagnostic methods for this disease [1].

IAAM Animal Models: The objectives of this contract are to establish and standardize animal models of invasive aspergillosis that will allow reliable quantification of infectious burden and to define surrogate markers of infection and disease progression [1,3].

IAAM Goals: These studies are aimed at improving the diagnosis and therapy of invasive aspergillosis by providing the extended *Aspergillus* research community resources to address "key questions" in the field, including genetic approaches to diagnosis, animal model experiments and training to establish these approaches [1].

RESULTS

- Murine Model of IPA
  - Inbred (Balb/C) and outbred (ICR) mice were challenged by inhalation of a nebulized suspension of *Aspergillus* conidia (10^9CFU/ml).
  - Similar trends of survival after infection were seen in both groups. FIGURE 1A.
  - At set time points, pulmonary fungal burden was assessed by CFU/g. Fungal burden of the groups was similar at 1 hour (1h) and day 1 (D1) of infection. ICRs maintained similar levels of CFUs to D5 while Balb/Cs showed a 1 $\log_{10}$ decrease. On D7 a 2 $\log_{10}$ decrease from D1 was seen in both groups. FIGURE 1B.
- Guinea Pig model of IPA
  - Hartley Guinea Pigs (GP) were challenged with *Aspergillus* conidia as previously described.
  - 100 % mortality was achieved within 9 days in this model of infection (mean day of death 6.76 ± 0.18). FIGURE 2A.
  - CFU/g data showed an approximate 1.5 $\log_{10}$ decrease in pulmonary tissue burden through out the course of the study. FIGURE 2B.

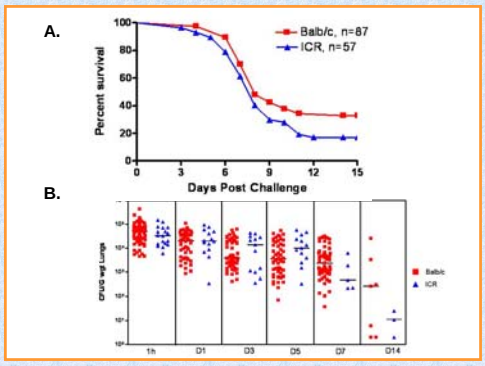

Figure 1. Survival and associated tissue burden in the lungs of Balb/c and ICR mice infected in the acrylic inhalation chamber.

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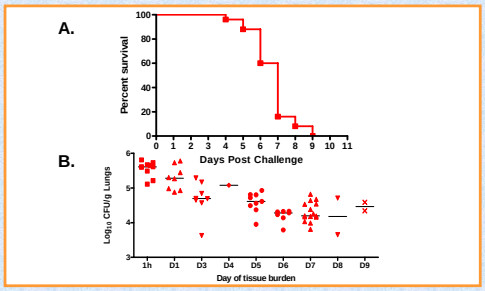

Figure 2. Survival and associated tissue burden in the lungs of Hartley guinea pigs infected in the acrylic inhalation chamber.

- Standardized model(s)
  - Recapitulate human disease
  - Reasonable costs
  - Reproducibility
  - Ease of use
  - Amenable to studies including:
    - Evaluation of novel diagnostics
    - Evaluation of host response
    - Evaluation of organism virulence factors through molecular manipulations
    - In vivo expression analysis
    - Evaluation of therapeutic compounds

TABLE 1. Objectives of standardized *Aspergillus* animal models

- Prioritization of key questions
  - High likelihood of commercialized diagnostic product
  - Data to support development of diagnostic product
  - Pilot studies to test theoretical diagnostic target
    - NIH funded research
    - Preliminary data to support NIH application with favorable priority score on review
    - Pilot studies to evaluate investigator initiated concept
    - Industry sponsored research

TABLE 2. Prioritization criteria for acceptance of key questions for use of the Contract's animal models

- Qualified investigator
  - Researcher with interest in *Aspergillus*
  - Trained to safely perform requested research
  - Qualifications: Principal investigators; Trainees (Post-doctoral fellows, students); Industry researchers
- Key Questions
  - Gene/gene product as diagnostic target
  - Evaluation of surrogate marker(s)
  - Effects of therapy on disease progression and / or gene expression
  - Role of virulence determinants in diagnosis
  - Others

TABLE 3. Examples of investigator's qualifications and potential key questions to be examined under the IAAM contract

CONCLUSIONS

- **Similar trends of survival were seen with both inbred and outbred mice in the inhalational murine model of IPA.**
- **CFU assessment of lung tissue burden shows uniformity of infection in both inbred and outbred mice.**
- **Data from both strains of mice were comparable therefore, the inexpensive ICR mice may be substituted in this model.**
- **A lethal inhalational GP model of IPA was obtained.**
- **CFU assessment of GP pulmonary tissues shows steady decrease in burden by study's end.**

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