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Design and evaluation of an aerosol infection chamber for small animals

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Abstract

In this report, we describe the design of an aerosol exposure chamber to reproducibly produce uniformly distributed clouds of droplet nuclei. The device can deliver desired number of bacilli (20–2000) in lungs of mice. All safety measures to handle infectious bacteria have been incorporated in the design and it is controlled remotely by a personal computer. It is an indispensable device to study the protective efficacy of vaccine candidates against *Mycobacterium tuberculosis* infection. This device would also be useful to study immunization and drug delivery by nasal route in experimental animals.

Keywords: Aerosol exposure; Infection chamber; *Mycobacterium tuberculosis* infection; Immunization; Drug delivery; Nasal route

1. Introduction

Over the past several years there has been a concerted effort to develop a new vaccine against tuberculosis (Lowrie et al., 1999; Orme, 1999; Kaufmann, 2000). The existing vaccine, *M. bovis* BCG has revealed lack of effectiveness in adults (Colditz et al., 1994). New vaccine candidates are tested in standardized animal models like mice and guinea pigs to determine if they have any protective activity against virulent *Mycobacterium tuberculosis* (TB) infection. Commonly, parenteral route is used to deliver mycobacteria rapidly into the blood stream like intravenous or intraperitoneal injection. These methods do result in a hematogenous seeding of the lung, but the timing of the arrival of blood-borne bacilli, which is the critical feature of TB pathogenesis, is not reproduced if the circulating organisms arrive in the lungs, weeks before the animal has developed cell-mediated immunity (Orme and McMurray, 1996). Thus, the parenteral injection of massive doses of virulent tubercle bacilli does not represent a rational approach to model human TB. This process bears no resemblance to the extrapulmonary dissemination that occurs following respiratory infection (McMurray et al., 1996).

Infection by respiratory route is the only relevant way to model pulmonary tuberculosis. The disease that results from low-dose, pulmonary exposure to virulent mycobacteria exhibits many important features of human TB (Baldwin et al., 1998; McMurray et al., 1999; McMurray, 2001). Unfortunately, the devices to infect animals by respiratory route in a controlled and reproducible manner are not commonly available. Here, we describe the design of an aerosol exposure chamber to produce uniform clouds of droplet nuclei, which result in the reproducible pulmonary infection to a group of mice simultaneously. In this animal infection chamber, all safety measures to handle infectious bacteria have

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been incorporated and it is remotely controlled by a personal computer, which further eliminates the risk of exposure of pathogenic bacteria to the researcher.

2. Materials and methods

2.1. Components of animal infection chamber

A schematic diagram of infection chamber is shown in Fig. 1. The chamber has following components.

2.1.1. The air tight chamber

The infection chamber is made from a box of transparent polycarbonate of size 30 cm × 20 cm × 20 cm. This box can accommodate up to ten mice conveniently. It has a transparent lid, which has connectors for aerosol inlet and exhaust. To ensure uniform distribution of aerosol in the chamber, three 12 V, 40 CFM, $3\frac{1}{8}$ in. size muffin fans (Rexnord, India) are mounted on the inner side of the lid. These fans also help in quick drying of liquid vapor and bacteria are inhaled as droplet nuclei. In order to make chamber air tight, a rubber gasket is placed in between the lid and the box, which are then firmly held together by 'nuts and bolts' or 'hard spring steel clamps'.

2.1.2. Aerosol generator

Respirable aerosol of the bacterial suspension is made by passing compressed air through a nebulizer holding the suspension. We have used a typical nebulizer commonly used for medication (Voyage, Mefar, Italy). This nebulizer is designed to generate respirable aerosol of 5–8 μm size. Antifoam A (Sigma Chemical Co., St. Louis, MO) is added to the bacterial suspension at a concentration of 0.5 μl/ml to match the fluid characteristics of bacterial suspension with the drug formulation for which the common nebulizers are designed.

2.1.3. Incinerator and air filter

The aerosol coming out of the chamber is made to pass through an incinerator. The incinerator is made from a double walled glass barrel with nichrom wire (2 kW, 220 V), wound in between two glass cylinders. The exhaust of infection chamber, is made to flow over red hot nichrom wire. After incineration the exhaust is bubbled through disinfecting fluid and finally passed through a 0.22 μm air filter (Millipore), before it is released in exhaust of Biosafety hood.

2.1.4. Computer control of components

To ease the operation and to make it more reliable, the assembly is controlled by wire remotely. A three way solenoid air valve, type L414 by Lesman USA, is used to join the air pump, nebulizer and the infection chamber. This is a standard 24 V, dc operating valve. This valve could either connect air pump and infection chamber directly, to flush the aerosol out of chamber or pass air through the nebulizer to fill the infection chamber with aerosol. All the electrical components, the fans, the air pump, the solenoid air valve and the nichrom wire incinerator are wired through respective relays. These relays are selectively actuated through a control panel placed at a distance from the infection chamber. This control panel is also connected to the parallel port of a personal computer (IBM 486, running under MS-DOS). Conventional parallel port communication protocol is used to control various relays and time duration (Dhananjay et al., 1994). This port allows the input of up to 9 bits or the output of 12 bits at any one given time. Various relays to switch off/on air pump, fans, solenoid, etc. are wired through these output bits which are set or reset by writing appropriate byte at the parallel port. More details of the circuit and the program may be obtained from the author.

2.2. Placement and working of the whole assembly

In order to ensure double safety to the experimenter, the device is placed in a Biosafety hood and controlled remotely. An algorithm of a typical experiment is given in Table 1. It takes around 30 min to complete one cycle of infection experiment. After the experiment, all the connecting tubes, nebulizer, chamber along with fans are autoclaved and reused (at least 10 times) or may even be discarded as the cost of all these is quite nominal. The cost of the materials of this infection chamber, excluding the PC, is around USD 250.

2.3. Bacterial culture

Mycobacterium.w (*M.w*) was grown in Middlebrook 7H9 media (Difco Lab, Detroit) for 7–10 days.

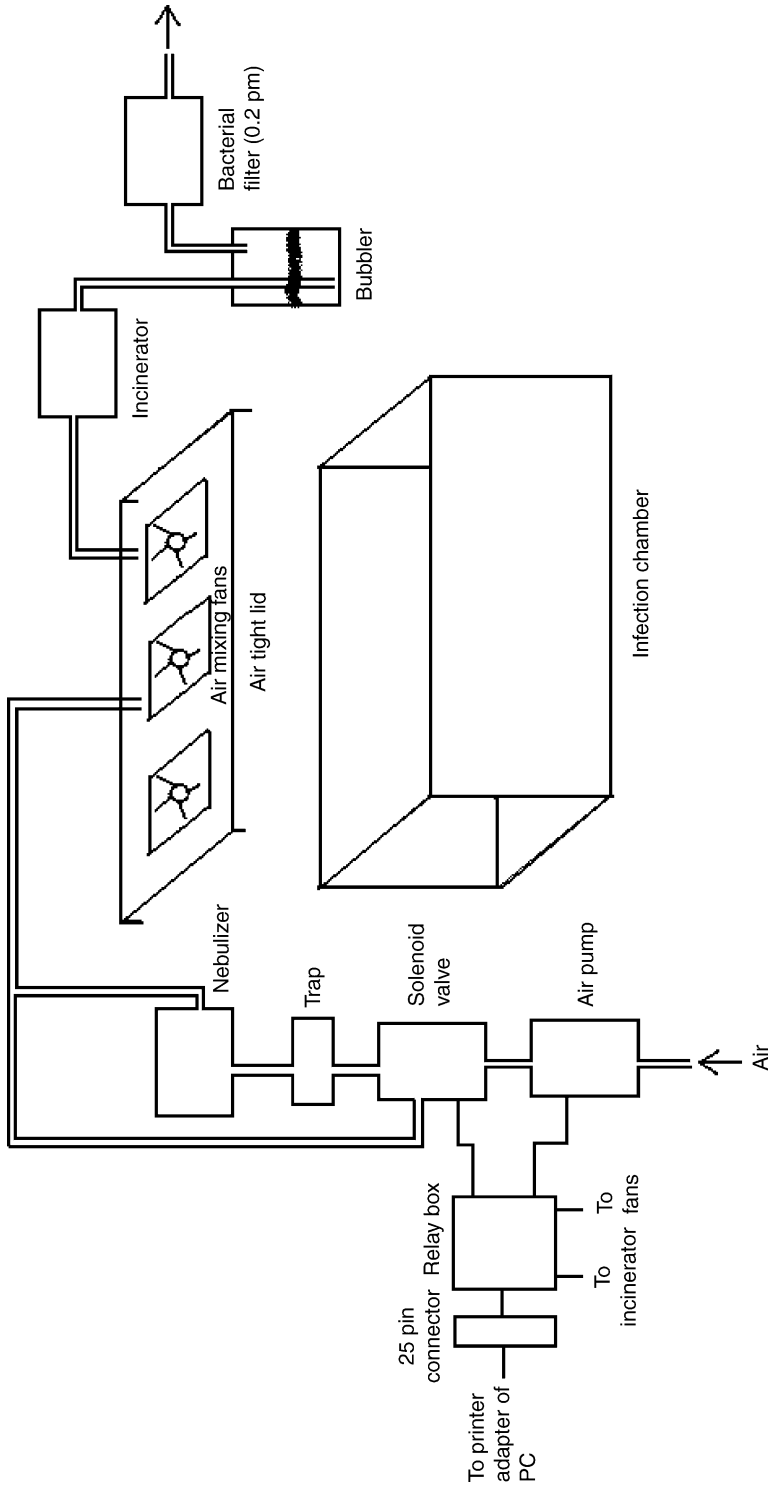

Fig. 1. Schematic diagram of small animal infection chamber.

Table 1
An algorithm of a typical experiment

Step	Activity	Setting on the control panel
1	Instrument check	Check (a) air tight seal of the chamber, (b) incinerator heating coil, (c) fans, (d) air pump, (e) solenoid air valve
2	Flush the chamber by clean air for some time, typically 5 min	Switch off incinerator heating coil, shunt the nebulizer, switch on fans, switch on air pump
3	Place animals inside the chamber	Switch off fans and air pump, replace the lid, again check the air tight seal
4	Flush the chamber by clean air for some time, typically 5 min	Switch off incinerator heating coil, shunt the nebulizer, switch on fans, switch on air pump
5	Generate aerosol for some time and let the mice inhale aerosol for a given period of time	Switch on the incinerator heating coil, actuate the solenoid air valve, switch on fans, switch on air pump
6	Flush the chamber by clean air for some time, typically 5 min	Switch on the incinerator heating coil, shunt the nebulizer, switch on fans, switch on air pump
7	Remove animals from the chamber	Switch off incinerator heating coil, shunt the nebulizer, switch off fans, switch off air pump

Bacilli were harvested in the log phase of growth and washed twice with sterile 0.15 M NaCl solution. The viable units were determined by counting colony forming units at appropriate dilutions of mycobacteria inoculated on to L–J (Lowenstein–Jensen) plates. To ease the process of counting for future experiments, calibration of OD at 600 nm versus number of bacilli/ml based on colony forming units (CFUs) on L–J plates was done. The function relating the OD and the concentration of bacilli in suspension was found to be, $\log(\text{bacilli suspension/ml}) = 0.484 + 5.36 \times \text{OD}_{600\,\text{nm}}$. Similarly, *Escherichia coli* DH5$\propto$ was grown in LB media overnight and washed twice with sterile 0.15 M NaCl solution before use in the experiment.

2.4. Animal experiments

Different groups of mice (C57BL/6, inbred strain of mice) were exposed to aerosol generated from different concentrations of bacteria in the suspension held in the nebulizer. They were exposed to aerosol of $M.w$ bacteria for 5 min. Twenty-four hours after exposure to aerosol, mice were killed by exposure to CO_2. Lungs were removed and homogenized in Teflon-glass homogenizer. These were homogenized in 0.5% albumin (bovine serum albumin, fraction V, Sigma) in sterile 0.15 M NaCl solution. Appropriate dilutions of tissue homogenates (neat, 1:10, 1:100, 1:500 and 1:1000) were streaked in duplicate over L–J plates. All plates were incubated at 37 °C. CFUs were counted after 8 days of incubation.

3. Results

3.1. Evaluation of function of aerosol assembly

3.1.1. Sterility of the air at the outlet

The device was checked for sterility of air coming out of it by generating an aerosol of *E. coli* and exposing the LB agar plates to outlet of the assembly. *E. coli* aerosol was generated for 10 min and a plate was exposed to the outlet for the entire period of experiment, including initial air flushing (5 min), aerosol generation (10 min) and final air flushing (5 min). Plates were incubated at 37 °C for 24 h. No *E. coli* colonies were seen on plates which confirms that air coming out of the aerosol chamber becomes sterile by the series of traps used.

3.1.2. Distribution of aerosol in the chamber

Uniform distribution of aerosol within the chamber was checked by making aerosol of *E. coli* DH5$\propto$ suspension. LB Agar plates were kept at different corners and center of the chamber. Aerosol of *E. coli* suspension was passed through the chamber for 5 min. Plates were taken out and incubated at 37 °C overnight. Uniform distribution of colonies was observed in all the plates.

3.2. Standardization of inhaled bacilli

3.2.1. Variation of the number inhaled bacilli with different concentrations of bacterial suspension

Different concentrations of bacterial suspension in the range $3 \times 10^4 - 3 \times 10^8$ per ml were taken in the

Table 2
Results showing number of bacilli inhaled with different concentrations of bacterial suspension taken in the nebulizer ($n = 2$)

Concentration of bacterial suspension taken in the nebulizer (bacteria/ml)	Number of bacteria inhaled in 5 min exposure (bacteria/mice)		
	Mice 1	Mice 2	Average
3.0×10^8	>2000	>2000	–
3.0×10^6	155	105	130
3.0×10^5	12	16	14
3.0×10^4	No colony seen on plate		

nebulizer, and the number of bacilli inhaled were estimated. Results are shown in Table 2. It was found by counting the CFUs of lung homogenates that concentration of 3.0×10^6 bacteria per ml in bacterial suspension placed in nebulizer deposits about 150 bacilli in lungs of mice in 5 min exposure time.

3.2.2. Variation of the number inhaled bacilli among different animals in one experiment

In order to establish the reproducibility of inhaled bacilli by different animals in one experiment, eight mice were caged in the chamber and a suspension of 3×10^6 bacteria per ml was taken in the nebulizer. All the mice were exposed to aerosol for 5 min. The number of inhaled bacilli were found to be 138 ± 12 per mice.

3.2.3. Variation of the number of inhaled bacilli in different set of experiments

Four independent experiments were performed to establish the reproducibility of inhaled bacilli in different experiments. In each experiment three mice were caged and a suspension of 3×10^6 bacteria per ml was taken in the nebulizer. In all the four experiments mice were exposed to aerosol for 5 min. Results are shown in Table 3. These values are not statistically different at 0.05 level around the mean.

Table 3
Results showing number of bacilli inhaled in four different experiments keeping the concentration of suspension (3×10^6 bacteria per ml) and exposure time (5 min) same

Experiment no.	Number of inhaled bacilli per mice
1	123 ± 25
2	145 ± 12
3	128 ± 20
4	119 ± 21

For all experiments ($n = 3$).

4. Discussion

The simple and inexpensive design of this aerosol device makes it very useful to study any kind of aerosol delivery. In this device, it is possible to vary pathogen concentration and length of exposure to deliver the optimal dose. Necessary safety measures, computer controlled remote operation and disposable nature of its components are the added advantages to infect animals from aerosol of pathogens using this device.

Not many devices exist for infecting experimental animals by the aerosol of pathogens. We have come across only one such apparatus available commercially, made by Glas Col, USA. In most of the other designs, the movements of animals are restrained and aerosol is delivered to the nose. Such designs are useful for delivering 'non-toxic' drugs but cannot be used for generating the aerosol of pathogens and other toxic substances as they lack necessary safety measures (Pettis et al., 2000; Sharma et al., 2001).

The device described in this paper can be used to infect experimental animals with *M. tuberculosis* by respiratory route which is the only relevant way to model tuberculosis (Baldwin et al., 1998; McMurray et al., 1999; McMurray, 2001). The disease that results from low-dose, pulmonary exposure to virulent mycobacteria exhibits many important features of human TB. Hence, this device will have application in studying the protective efficacy of new candidate vaccines for tuberculosis.

Due to the reproducibility in delivering number of aerosol droplets to the experimental animals, this device would also be useful for studies involving drugs delivery or immunization by nasal route (Falero-Diaz et al., 2000; Quadir et al., 2000). Intranasal (i.n.) immunization in man offers distinct advantages over the oral or parenteral route of vaccination against infections at both local and distant sites. In experimental

animal models, i.n. vaccination has shown pronounced mucosal IgA, as well as systemic IgG, Th1 and Th2 responses against aerogenic challenge with a number of bacterial pathogens of the upper and lower respiratory tract (Lagranderie et al., 1993; Gheorghiu, 1994). This route of vaccination is of active interest in endeavors to improve the efficacy of vaccination against a number of respiratory infections. The induction of mucosal immune responses is particularly important for protection against diseases for which entry and pathogenesis are related to the mucosal system, such as salmonellosis, AIDS and tuberculosis (Gustavo et al., 2000).

Besides vaccines, the simplest and most natural route of drug delivery to the lungs is by inhalation for the treatment of respiratory diseases. Large highly absorptive surface area of the lung could also be used for systemic delivery of proteins such as insulin (Davis, 1999). Even small molecular weight drugs are tested via the inhalation route either to provide non-invasively rapid onset of action, or to improve the therapeutic ratio for drugs acting in the lung (Gonda, 2000; Sharma et al., 2001).

This assembly would be of use to study the protective efficacy of vaccine candidates against *M. tuberculosis* infection and for studies involving development of many valuable therapeutic products to be delivered by inhalation.

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