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Age-related differences in the neutrophil response to pulmonary pseudomonas infection

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ABSTRACT

Background: Pseudomonas aeruginosa pneumonia is more common and more lethal in the elderly. The immunologic underpinnings of this increased incidence and mortality have not been evaluated, however are assumed to be a complication of age-associated immune dysfunction.
Methods: Young (10–12 week old) and aged (18–20 month old) BALB/c mice were subjected to intratracheal infection of *P. aeruginosa*. Animals were sacrificed 24 h after inoculation. The lungs were collected for analysis of lung pathology, chemokine levels, neutrophil counts, and myeloperoxidase activity.
Results: Pulmonary levels of the neutrophil chemokine KC are significantly higher in aged mice relative to young following *P. aeruginosa* infection. Despite this, neutrophil counts are higher in young mice compared to aged mice after infection. Furthermore, the neutrophils are predominantly found in the air space of young infected mice. This correlated with increased myeloperoxidase activity from bronchoalveolar lavage specimens of young mice relative to aged mice after infection.
Conclusions: Neutrophil migration into the lungs is impaired in aged mice 24 h after intratracheal infection despite elevated chemokine levels, suggesting that immunosenescence is impairing neutrophil migration.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is the second most common bacterial organism to cause hospital-acquired and ventilator-associated pneumonia in the U.S. and worldwide, accounting for nearly one in five bacterial pneumonias (Jones, 2010). The incidence of *P. aeruginosa* pneumonia increases with advancing age (Venier et al., 2011), as does its associated mortality (Tumbarello et al., 2013). This combination makes it a particularly problematic disease in the elderly. *Pseudomonas* pneumonias induce an intense pro-inflammatory response in the first 24 h after infection, largely characterized by neutrophil infiltration and activation (McConnell et al., 2010). This crucial early innate immune response depends not only on neutrophil activation and phagocytic ability, but also on mobilization and directed migration to the site of infection. Given the changes that occur to the immune system with advancing age (Plackett et al., 2004), it is likely that this immune response may be significantly altered in the elderly, accounting for their increased susceptibility to *P. aeruginosa* infections. A greater understanding of the mechanisms of age-related innate immune dysfunction in the setting of pneumonia is required to address this growing at-risk patient population. Clinical evidence and animal studies have repeatedly demonstrated diminished neutrophil function with advanced age though the precise mechanisms of neutrophil impairment may depend on the context and nature of the insult. The studies herein investigate the neutrophilic response in aged and young mice to the clinically relevant setting of a pulmonary infection with *P. aeruginosa*.

2. Methods

2.1. Animals

Young (10–12 weeks) and aged (18–20 months) female BALB/c mice were obtained from the National Institute of Aging colony at Charles River Laboratories (Wilmington, MA) and allowed to acclimate at the Loyola University Chicago Animal Care Facility. Mice were housed under specific pathogen-free conditions on a 12-hour light and dark cycle with free access to food and water. All animal studies were performed with strict accordance to and approval of Loyola's Animal Care and Use Committee.

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2.2. Intratracheal infection

Mice were anesthetized with an intraperitoneal injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). Animals then received an intratracheal inoculation with *P. aeruginosa* (ATCC #19660) as previously described (Davis et al., 2004; Murdoch et al., 2008) with minor modifications. Briefly, mice were placed in a supine position and a small incision was made to expose the trachea. The inoculum was given by instilling 100 μL of a predetermined concentration of *P. aeruginosa* followed by 100 μL of air directly into the trachea. The survival analysis was performed using *P. aeruginosa* inoculums of 10,000, 40,000, and 100,000 colony forming units (CFU). All other analyses were performed using a concentration of 40,000 CFU of *P. aeruginosa*. The incision was closed with wound clips and the animals were placed on their abdomen on a 30° incline while recovering from anesthesia. Body temperature was maintained at physiologic levels by placing the cages on heating pads until the animals were fully awake and ambulating. Sham animals were treated in an identical manner except they were injected with 100 μL of sterile saline followed by 100 μL of air.

2.3. Circulating granulocyte counts

Blood was collected via cardiac puncture immediately after sacrifice and put into capillary tubes containing EDTA to prevent coagulation. Peripheral blood samples were then analyzed for differential blood counts using a Heska Multivet veterinary blood analyzer (Heska, Loveland, CO).

2.4. Pulmonary chemokine levels

Animals were sacrificed 24 h after infection. Necropsy was performed and animals with visible tumors were excluded from further analysis. The right lung lobes were removed and snap frozen in liquid nitrogen. The lung lobes were stored at −80 °C until ready for further analysis. Frozen tissue was homogenized in BioPlex cell lysis buffer (BioRad, Hercules, CA) according to the manufacturer's instructions and analyzed for chemokine (KC) with a BioPlex multiplex bead array according to the manufacturer's specifications. Results were normalized to total protein measured by the BioRad Protein Assay.

2.5. Histopathology

After sacrificing the animal, the upper left lung lobe was inflated with 10% formalin, fixed overnight and embedded in paraffin before being sectioned (5 μm) and stained with hematoxylin and eosin (H&E) as previously described (Patel et al., 1999). In a blinded fashion, 10 high power fields (400×) per animal were assessed for neutrophil infiltration into the pulmonary interstitium and air space.

2.6. Myeloperoxidase activity

To confirm neutrophil counts from the pulmonary interstitium and air space, myeloperoxidase (MPO) activity was determined in bronchoalveolar lavage (BAL) fluid and lavaged lung tissue. BAL fluid was collected by lavaging the lungs with five aliquots of 0.2 mL of RPMI-1640 media as described elsewhere (Davis et al., 2004). Lavaged lung lobes were homogenized in phosphate buffer containing 0.5% hexadecyltrimethylammonium. The samples were then sonicated and the supernatants cleared by centrifugation before incubation with *o*-dianisidine hydrochloride and hydrogen peroxide. MPO content in the samples was determined based on optical density readings from the MPO standard (Sigma Aldrich, St Louis, MO), which was run in parallel. Results were normalized to the total protein content of each sample. Data are listed as MPO activity per mg of protein.

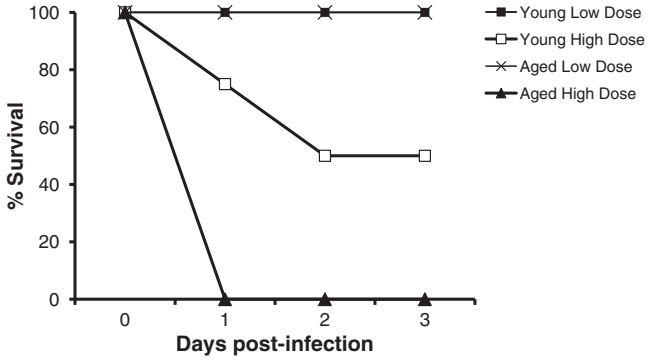

Fig. 1. Survival after intratracheal infection with *Pseudomonas aeruginosa* at low dose (10,000 CFU) and high dose (100,000 CFU) inoculums. Saline control and mid dose (40,000 CFU) demonstrated 100% survival and is not shown.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences between young and aged mice with and without infection, and Tukey's post-hoc test once significance was achieved. A student's *t*-test was used to compare MPO levels between young and aged infected mice. A p-value of <0.05 was considered significant. Data are reported as mean values ± standard error of the mean.

3. Results

3.1. Survival

Optimization of the infection model was balanced to maximize the size of inoculum and maintain 100% survival of young and aged mice. Intratracheal administration of 10,000 CFU is associated with 100% survival of young and aged mice for the first three days after infection (Fig. 1). Likewise, 40,000 CFU was associated with 100% survival between both groups at 3 days after infection. Increasing the inoculum to 100,000 CFU is associated with 50% survival in young mice at 3 days after infection and yielded 100% mortality within 24 h after inoculation in aged mice.

3.2. Circulating granulocytes

A significant increase in circulating granulocytes was observed in aged mice given infection compared to saline-treated controls (Fig. 2). In comparison, no significant change was observed between young

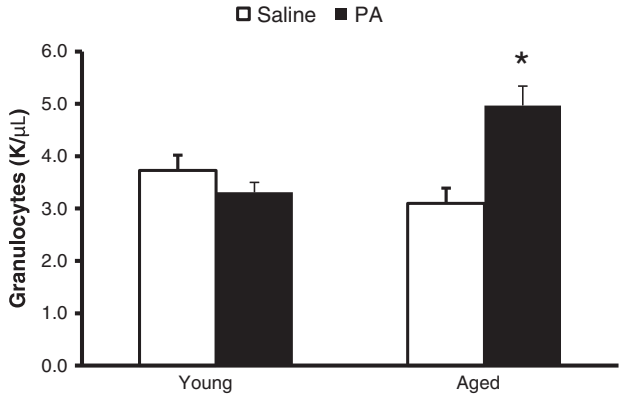

Fig. 2. Circulating granulocytes. Blood was collected after sacrifice and analyzed for number of granulocytes. N = 4 animals per groups. *p < 0.05 compared to young infected and aged saline treated mice.