Chlorine gas exposure increases susceptibility to invasive lung fungal infection

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EXPOSURE TO CHLORINE (Cl2), either through chemical disasters, such as railway spills, or passive exposure, such as inhalation of disinfectants, can result in profound changes in the lung. Even minute exposure to Cl2 (1–5 ppm) can lead to acute changes in forced expiratory volume at 1 s (FEV1) and functional residual capacity (FRC) (45). It is known that the extent of symptoms leading up to lung injury can vary depending on factors such as exposure concentration, exposure duration, ventilation, and host susceptibility (reviewed in Ref. 20). Although the most well studied effects of Cl2 exposure on lung physiology in humans have been in low-dose challenge studies or assessments within the first few days after acute exposure, the effects of Cl2 exposure on long-term lung complications have also been reported. Workers in various industrial settings that are chronically exposed to low-dose Cl2 reveal incidences of decreased pulmonary function, such as diffusing capacity and FEV1-to-FVC ratios, over time (reviewed in Ref. 20). Case reports have documented incidences of obstructive lung disease several years after a single Cl2 gas exposure (47, 48). The development of irritant-induced asthma, also called reactive airways dysfunction syndrome or RADS, is another reported long-term consequence of Cl2 gas exposure (12).

A commonality between obstructive airway diseases, such as chronic obstructive pulmonary disorder (COPD) and asthma, is that microorganisms may often contribute to the lung phenotype. With regard to COPD, lung exacerbations are often thought to be microbial in origin (50). Over a decade ago, the “vicious circle hypothesis” was proposed for COPD, which states that cigarette smoke impairs innate lung defenses, allowing microbial pathogens to become established in the lower respiratory tract, leading to chronic inflammation and lung destruction (49). In the context of asthma, exposure to or contamination of allergens with microorganism-derived compounds, such as LPS (from Gram-negative bacteria; Ref. 55) or fungal cell wall components (chitin, beta-glucans; Ref. 59), often leads to rapid lung responsiveness and reactions such as airway hyperreactivity. Moreover, reports indicate that, among severe asthmatics, sensitivity to fungi range from 25% to over 70% (reviewed in Ref. 19) and correlate with hospital/ICU admissions compared with asthmatics that do not require hospitalization (1). Since Cl2 gas exposure may lead to an obstructive lung disease (47, 48) or asthma-like phenotype (12) over time, it is conceivable that microbial colonization or infection may occur after Cl2 gas exposure.

According to the Office of The Surgeon General, United States Army (58), Cl2 gas exposure is of historical significance during wartime. Incidences of chronic bronchitis were thought to be common after World War I Cl2 inhalant exposures, which were thought to occur in association with pulmonary infections. It was further documented that chronic or progressive illness was more likely to have resulted from a combination of inadequately treated complicating infections and cigarette smoking. Finally, bacterial superinfection was commonly noted within 5 days postexposure to Cl2 (58). These observations suggest that lung infections are a possible consequence of Cl2 gas exposure. In the present study, we investigated the effects of Cl2 gas exposure on the lung immune response to Aspergillus fumigatus. A. fumigatus is a ubiquitous mold inhaled daily by humans that is normally cleared by the lung innate immune system. In susceptible individuals, however, A. fumigatus can cause life-threatening invasive fungal infections [invasive pulmonary aspergillosis (IPA)] (2, 30, 31). We show here that Cl2
gas exposure negatively affects cellular and inflammatory responses critical for the elimination of A. fumigatus from the lungs and results in significant increases of airway hyperreactivity and alveolar permeability to plasma proteins.

MATERIALS AND METHODS

Mice. C57BL/6 male mice (8 wk old, 20 g body wt) were purchased from Charles River Laboratories (Wilmington, MA). Mice were maintained in a specific pathogen-free environment in microisolator cages within an American Association for Laboratory Animal Science-certified animal facility in the Bevill Biomedical Research Building II at the University of Alabama at Birmingham. Animal studies were reviewed and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC).

Cl2 gas exposure. Mice were exposed to Cl2 gas (400 ppm) in a cylindrical glass chamber for 30 min, as previously described (34, 52, 67, 71), and returned to room air. Continuous measurements of Cl2 concentrations during the exposure were monitored with an Interscan (model RM34-1000 m) Cl2 detector, connected to a data logger for data storage.

Preparation of A. fumigatus, in vivo challenge and lung fungal burden assessment. A. fumigatus isolate 13073 (ATCC, Manassas, VA) was maintained on potato dextrose agar for 5–7 days at 37°C. Conidia were harvested by washing the culture flask with 50 ml of sterile PBS supplemented with 0.1% Tween-20. The conidia were then passed through a sterile, 40-μm nylon membrane to remove hyphal fragments and enumerated on a hemocytometer. Twenty-four hours post-Cl2 exposure, mice were lightly anesthetized with isoflurane and administered 7 x 107 A. fumigatus conidia in a volume of 50 μl intratracheally. Briefly, mice are held in a vertical, upright position, and the tongue is withdrawn from the mouth using forceps. A pipette is used to deliver the 50 μl of inoculum to the caudal oropharynx in which normal breathing results in fluid aspiration into the lungs (41). Controls included mice exposed to Cl2 and administered PBS intratracheally and mice exposed to air and then challenged with A. fumigatus. For lung fungal burden analysis, the left lungs were collected and homogenized in 1 ml of PBS. Total RNA was extracted from 0.1 ml of unclarified lung homogenate using the MasterPure yeast RNA purification kit (Epicentre Biotechnologies, Madison, WI), which includes a DNAse treatment step to eliminate genomic DNA as previously reported (36). Total RNA was also extracted from serial 1:10 dilutions of live A. fumigatus conidia (104 to 109) and DNAs treated to form a standard curve. Lung A. fumigatus burden was analyzed with real-time PCR measurement of the A. fumigatus 18S rRNA [GenBank accession no. AB008401 (11)] and quantified using a standard curve of A. fumigatus conidia as previously described (36). As a validation of the real-time PCR method, heat-killed A. fumigatus did not yield a signal by real-time PCR and were unable to grow on potato dextrose agar plates (36). In addition, no amplification controls (i.e., no reverse transcriptase included in the cDNA reaction) yielded a signal of <0.001% by real-time PCR, indicating that the DNase treatment step was efficient at eliminating contaminating A. fumigatus DNA [since DNA is not predictive of organism viability (27)].

Lung injury, inflammatory cell, and lung function analysis. For lung injury analysis, 72 h post-A. fumigatus challenge, a bronchoalveolar lavage (BAL) was performed as previously described (34, 52, 67, 71). The BAL fluid was spun at 150 g for 10 min at 4°C to pellet cells and cellular debris. Protein concentrations in cell-free BALF samples were measured with the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL) using the microtiter plate protocol as previously described (34, 52, 67, 71). Equal volumes of BAL fluid were separated by denatured SDS-PAGE (10%) and transferred to polyvinylidene difluoride (PVDF) membranes and immunoblotted for murine albumin by using goat anti-mouse albumin (GeneTex, Irvine, CA) and anti-goat IgG-horseradish peroxidase (HRP; Santa Cruz Biotechnology, Dallas TX) or murine IgG using chicken anti-H+M+R IgG Fc (Abcam, Cambridge, MA) and rabbit anti-chicken IgY-HL-HRP (Abcam, Cambridge, MA). Protein bands were revealed by enhanced chemiluminescence (Pierce Biotechnology, Rockford, IL) and exposed to X-ray films. For assessment of inflammatory cells, live and dead cells collected from BAL fluid were enumerated using trypan blue staining. Cell differential counts were determined from 300 live cells per cytospin slide, which were prepared using a cytospin centrifuge (Shandon, Pittsburgh, PA) and stained with Wright protocol (Kalamazoo, MI).

For measurement of airway reactivity and lung resistance and elastance, mice were anesthetized with an intraperitoneal injection of Nembutal (sodium pentobarbital; 50 mg/kg body wt), paralyzed with an intraperitoneal injection of Pavulon (pancuronium bromide; 2 mg/kg body wt), intubated, connected to a ventilator (Flexivent; SCIREQ, Montreal, Canada), and ventilated at a rate of 160 breaths/min at a tidal volume of 0.2 ml with a positive end-expiratory pressure of 3 cmH2O. Newtonian resistance, total respiratory system resistance, and elastance were recorded continuously, as previously described (52, 61). Baseline was set via deep inhalation. Increasing concentrations of methacholine chloride (0–40 mg/ml; Sigma-Aldrich, St. Louis, MO) were administered via aerosolization within an administration time of 10 s. Airway responsiveness was recorded every 15 s for 3 min after each aerosol challenge. Broadband perturbation was used, and impedance was analyzed via constant phase model.

Whole lung cytokine and chemokine analysis. Seventy-two hours post-A. fumigatus exposure, the left lung was excised homogenized in PBS supplemented with Complete Mini protease inhibitor tablets (Roche), clarified by centrifugation, and stored at −80°C. Supernatants from lung homogenates were analyzed for protein levels of 23 cytokines and chemokines using Bio-Plex multiplex suspension cytokine array (Bio-Rad Laboratories), according to the manufacturer’s instructions (26, 61). The data were analyzed using Bio-Plex Manager software (Bio-Rad Laboratories). IL-23 and IL-22 levels were quantified by ELISA (R&D Systems), as previously described (26, 61).

Assessment of rates of superoxide production by lung inflammatory cell levels. The rate of superoxide (O2−) generated by inflammatory cells in the BALF was measured by monitoring the reduction of cytochrome c (23) with a Shimadzu UV-2501PC spectrophotometer (Kyoto, Japan) at 37°C. A BAL was performed on air or Cl2-exposed mice 72 h post-A. fumigatus challenge, and lavaged inflammatory cells were resuspended in 1 ml of PBS and kept at 4°C until the time of the experiment. One milliliter of buffer (10 mM potassium phosphate with 100 μM DTPA at a pH of 7.4) and 50 nM of cytochrome c (Sigma-Aldrich, St. Louis, MO) containing 2 x 105 cells was added to a spectrophotometer cuvette. The reference cuvette contained buffer and cytochrome c in the absence of cells. Both cuvettes were heated to 37°C. Absorbance at 550 nm was continuously recorded for 3 min. At this time, PBS or 100 mg of phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) in DMSO was added into the reference and measurement cuvettes, respectively, and absorbance was measured for ~5 min. Superoxide dismutase (SOD; 200 U; Sigma-Aldrich) and catalase (250 U; Sigma-Aldrich) were then added in both cuvettes, and absorbances were measured for an additional 5 min. Bone marrow-derived neutrophils were provided courtesy of Dr. Jaroslaw Zmijewski (Department of Medicine, University of Alabama).

Statistics. Data were analyzed using GraphPad Prism version 5.0 statistical software. Comparisons between multiple groups with data were normally distributed were made with one-way ANOVA and with Student’s t-test when two groups were compared. Significance was accepted at a value of P < 0.05.

RESULTS

Compromised lung microbial clearance after Cl2 exposure. Although the effects of Cl2 gas exposure on lung inflammation, airway epithelial cell injury, and pulmonary edema are well

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Cl2 gas exposure results in sustained impairment in lung microbial clearance.

Increased lung injury in Cl2-exposed mice after microbial challenge. To determine whether Cl2 exposure followed by microbial challenge resulted in increased injury to the alveolar epithelium and microvascular endothelium, we assessed the levels of serum albumin and IgG in BALF by Western blotting (34). Representative blots are shown in Fig. 2, A and B, and cumulative data of mean densitometry values are presented in Fig. 2, C and D. We have previously reported that exposure of mice to 400 ppm of Cl2 for 30 min leads to increased concentrations of albumin and IgG in the BAL at 30 min and 24 h postexposure (34). Data shown in Fig. 2 indicate that, at 72 h postexposure, BAL albumin and IgG are slightly elevated or at control levels, indicating that Cl2-induced injury to the blood gas barrier was in the early stages of repair. A. fumigatus challenge resulted in significant increases in albumin (Fig. 2, A and C) and IgG (Fig. 2, B and D) levels in BALF compared with mice exposed to air. However, mice exposed to Cl2 followed by A. fumigatus challenge demonstrated the highest amount of albumin (Fig. 2, A and B) and IgG (Fig. 2, C and D) in BALF, suggesting that microbial infection post-Cl2 exposure results in increased lung injury consistent with the development of pulmonary edema.

Impaired pulmonary function in Cl2-exposed mice after microbial challenge. Since data in Fig. 2 suggested increased lung injury in mice challenged with A. fumigatus after Cl2 exposure, we determined the magnitude this translated into decrements in lung function. We show that mice challenged with A. fumigatus after Cl2 exposure demonstrated higher total lung resistance (Fig. 3A) and elastance (Fig. 3B) before and after challenge with methacholine, as opposed to mice exposed to either Cl2 or A. fumigatus alone. Intriguingly, major airway resistance, although trending higher, was not significantly increased.

A. fumigatus. Thus Cl2 gas exposure results in sustained impairment in lung microbial clearance.

Results in Fig. 1 show that, 72 h after challenge, mice exposed to Cl2 had a >3-log increase in live fungal organisms compared with mice exposed to room air and challenged with A. fumigatus alone. Intriguingly, major airway resistance, although trending higher, was not significantly increased.

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increased in mice challenged with A. fumigatus after Cl2 exposure (Fig. 3C). Thus, coupled with the lung injury data in Fig. 2, lung infection after Cl2 exposure increases airway and alveolar epithelial injury and impairs lung function consistent with the development of reactive airway disease syndrome.

Cl2-exposed mice demonstrate intact lung myeloid cell recruitment but impaired antimicrobial activity after microbial challenge. A component of the lung response to acute Cl2 gas exposure is the recruitment of neutrophils in the lung interstitial and alveolar spaces (67). Although often viewed as a contributor to lung injury, neutrophils are essential for the clearance of pathogens from the lung, including A. fumigatus (6, 24, 53). We determined whether microbial challenge after Cl2 gas exposure modulated the recruitment of inflammatory cells, including neutrophils. Mice were exposed to Cl2 and A. fumigatus as described in Fig. 1, and inflammatory cell numbers in lung lavage fluid were quantified by differential staining. Results in Fig. 4A show that recruitment of myeloid cells in response to microbial challenge is not impaired by Cl2 gas exposure. In fact, Cl2 exposure followed by A. fumigatus challenge resulted in significantly higher recruitment of lymphocytes, monocytes, and neutrophils to the lungs compared with Cl2 exposure alone. However, since Cl2-exposed mice were unable to clear A. fumigatus yet neutrophils were present in high numbers, we determined whether the recruited inflammatory cells produced reactive oxygen species (ROS), which are essential for neutrophil antifungal activity against A. fumigatus (22, 43). Results in Fig. 4B (representative data) and Fig. 4C (cumulative data) show that, despite functional migration to the lungs of Cl2-exposed mice challenged with A. fumigatus, inflammatory cells had a profound impairment in ROS production as measured by cytochrome c reduction. In fact, bone marrow-derived neutrophils treated with PMA reduced cytochrome c 10 times faster than myeloid cells (~60% neutrophils) in the BALF fluid of mice breathing air and challenged with A. fumigatus (data not shown). Thus Cl2 gas exposure negatively affects inflammatory cell/neutrophil antimicrobial activity in mice exposed to a pathogenic microbe.

Comprehensive assessment of proinflammatory cytokine and chemokine levels in the lungs of Cl2-exposed mice after microbial challenge. As stated earlier, Cl2 gas exposure induces inflammatory changes in the lung, which is often characterized by the recruitment of neutrophils and induction of such cytokines as IL-6, TNF-α, and CXCL1/KC (52, 56). We therefore questioned whether these proinflammatory mediators were modulated by Cl2 after microbial challenge. Results in Fig. 5 show that Cl2 did not have a negative effect on the production of IL-1α, IL-1β, IL-6, and TNF-α after A. fumigatus challenge (Fig. 5A). Similarly, robust CXCL1/KC, CCL2/MCP-1, and CCL3/MIP-1α chemokine production was observed in Cl2-exposed mice after microbial challenge (Fig. 5B), most likely as a result of higher fungal burden in the Cl2-exposed group (Fig. 1). In contrast, the levels of IL-12p40, which is a component of the cytokines IL-12p70 and IL-23, was significantly decreased by Cl2 gas exposure (Fig. 5C). G-CSF is a potent growth factor for neutrophil survival and mobilization from the bone marrow (18) and has been shown to augment neutrophil-mediated killing of A. fumigatus (35). Intriguingly, G-CSF levels after microbial challenge were not impaired by Cl2 exposure (Fig. 5C). Thus Cl2 gas exposure does not impair proinflammatory cytokine and chemokine production after microbial challenge, yet it does inhibit the production of specific myeloid-derived cytokines, such as IL-12p40.

Impaired induction of neutrophil-derived IL-17A and IL-22 in Cl2-exposed mice after microbial challenge. The inflammatory cytokines IL-17A and IL-22 are essential for host defense against multiple viral and bacterial lung pathogens (4, 5, 21, 33). A major function of IL-17A and IL-22 in the lung is the induction of epithelial antimicrobial responses (4). We have previously reported that lung clearance of A. fumigatus in vivo also requires IL-17A (61) and IL-22 (26). Moreover, we have reported that the lung cellular source of IL-17A and IL-22 during acute A. fumigatus infection is neutrophils (26, 62).
Finally, we have shown that optimal production of both IL-17A (62) and IL-22 (26) require IL-23, which is produced by dendritic cells in a Dectin-1-dependent manner. IL-23 is comprised of IL-12p19 and IL-12p40 (42). Since IL-12p40 was lower in Cl2-exposed mice after microbial challenge, we questioned whether Cl2 gas targeted the IL-23/IL-17A/IL-22 axis while leaving other inflammatory axes (such as chemokine production) intact. Results in Fig. 6A show that, 72 h after challenge and despite having significantly higher A. fumigatus...
lung burden (Fig. 1), mice exposed to Cl₂ had significantly lower IL-17A and IL-22 levels in the lungs compared with mice exposed to room air and challenged with A. fumigatus. Lower IL-17A and IL-22 72 h after challenge are likely a result of an early impairment in IL-23 production, since mice exposed to Cl₂ had significantly lower IL-23 levels 24 h, but not 72 h, after challenge (Fig. 6B). Thus Cl₂ exposure impairs the ability of neutrophils to produce IL-17A and IL-22, a major function of which is to induce the epithelial antimicrobial response.

**DISCUSSION**

Clinical evidence indicates that exposure to Cl₂ gas has the potential to induce a lung environment that is conducive to the development of infection (63). In fact, antibiotics are often prescribed to individuals exposed to Cl₂ as a prophylactic measure to control the development of infection (63). Assessing this experimentally, we show here that exposure to Cl₂ gas results in profound immune suppression leading to the development of an invasive fungal infection that is most often observed in individuals with defects in neutrophil function (6, 24, 53). Moreover, Cl₂-exposed mice challenged with A. fumigatus demonstrated greater lung injury and poorer lung function compared with Cl₂ exposure alone, indicating that exposure to microbes following an exposure to Cl₂ may exacerbate Cl₂-associated toxicity.

We first asked the question of whether exposure of mice to a dose of Cl₂ that induces lung injury (34, 52, 67, 71) rendered mice more susceptible to microbial challenge. For these studies, we chose the opportunistic fungal organism A. fumigatus, since it is ubiquitous in the environment, inhaled daily by humans, and causes infections only in those with severe immune suppression (2, 30, 31). Despite a Cl₂-exposure regimen that results in lung injury but rarely mortality, this level of exposure had a dramatic effect on antimicrobial lung clearance mechanisms. We quantified A. fumigatus lung burden via real-time PCR measurement of A. fumigatus 18S rRNA in lung tissue, which is the most sensitive method for the determination of lung fungal burden in experimental aspergillosis (51). Employing a standard inoculum of 70 million conidia, which is based on published reports employing inoculums at this concentration when fungal clearance in animals that are not chemically or genetically immunosuppressed is assessed (7, 8, 25) as well as our own work (26, 61), it is impressive to note that 3 days after challenge, Cl₂-exposed mice had the equivalent of 27 million live organisms in their lungs compared with only 49,000 live organisms in the lungs of mice exposed to air. This inability of mice exposed to Cl₂ to clear A. fumigatus led to decrements in lung physiological measurements. More specifically, compared with mice exposed to Cl₂ alone, lung resistance was enhanced in Cl₂-exposed mice challenged with A. fumigatus by 166% at baseline, which increased to 242% at the highest dose of methacholine challenge. Similarly, lung elastance was enhanced in Cl₂-exposed mice challenged with A. fumigatus by 153% at baseline, which increased to 224% after methacholine challenge. Further insight into decreased lung function was revealed by characterization of albumin and IgG levels in lung lavage fluid, which demonstrated much higher levels in Cl₂-exposed mice challenged with A. fumigatus. Collectively, Cl₂ gas exposure renders the lung susceptible to microbial infection, and this combination leads to accentuated pulmonary edema, lung leakage, and compromised respiratory mechanics.

Our data indicate that, in addition to severely injuring the lung, Cl₂ gas exposure compromises neutrophil defense mechanisms, leading to impaired antimicrobial immunity. Neutrophil deficiency/dysfunction is the hallmark predisposing factor for the development of invasive fungal infection caused by A. fumigatus (6, 24). However, recruitment of neutrophils to the lung is a hallmark of Cl₂ gas exposure (16, 57, 71). Indeed, we observed that Cl₂-exposed mice challenged with A. fumigatus had much higher neutrophil levels than either air-exposed mice challenged with A. fumigatus or mice exposed to Cl₂ alone. This heightened recruitment of neutrophils directly correlated with enhanced production of CXCL1/KC and CCL3/MIP-1α, two chemokines documented to play a critical role in the recruitment of neutrophils to the lungs during A. fumigatus infection (38, 39). Collectively, these observations suggest that Cl₂ gas exposure does not impair the generation of pro-neutrophil chemokine production nor does it inhibit the ability of neutrophils to respond to chemotactic signals induced by a lung microbial infection.
Neutrophils kill *A. fumigatus* through multiple mechanisms, including oxidative mechanisms, such as ROS (43) and MPO (3), and nonoxidative mechanisms, such as lactoferrin (70), pentraxin 3 (28), and calprotectin (9). Of these, ROS, and specifically superoxide, is considered the most indispensable for the killing of *A. fumigatus* by neutrophils. Indeed, NADPH oxidase deficiency in humans, i.e., chronic granulomatous disease, is uniquely associated with the development of IPA (22). Moreover, mice deficient in the NADPH oxidase subunits gp91 or p47 are arguably the most susceptible mouse strain for the development for experimental *A. fumigatus* infection (40, 43). Although neutrophil recruitment was not impaired in Cl2-exposed mice challenged with *A. fumigatus*, these mice had significantly higher lung burden compared with air-exposed mice challenged with *A. fumigatus*. Since neutrophils are required for the elimination of *A. fumigatus* from the lungs in humans and experimental animal models (6, 24, 53), the probability was high that Cl2 exposure results in defects in neutrophil-mediated defenses. Indeed, we further discovered that inflammatory cells (nearly two-thirds of which were neutrophils) isolated from the lungs of Cl2-exposed mice challenged with *A. fumigatus* were incapable of producing ROS. Since macrophages from mice deficient in NADPH oxidase do not have impaired killing activity against *A. fumigatus* (10, 40), these results suggest that impaired ROS production by neutrophils contributes to impaired lung clearance of *A. fumigatus* in Cl2-exposed mice. It would be important in future studies to determine the extent by which Cl2 affects assembly of the NADPH oxidase complex in neutrophils. Although neutrophils are often documented to be part of the lung response to Cl2 gas exposure, and thus deemed a harmful contributor to lung injury, experimental studies have shown that some therapeutic interventions after Cl2 exposure may ameliorate lung injury and/or lung function without having a significant impact on neutrophil numbers (16, 52, 67). This would suggest that some aspects of Cl2-induced lung injury are not mediated by neutrophils. In turn, our data suggest that, after Cl2 gas exposure, neutrophils have an ability to respond to chemokines yet an inability to respond to microbes via the production of ROS. This defect in host defense leads to increased susceptibility to infection or long-term microbial colonization.

Dectin-1 is a 43-kDa, type II transmembrane receptor containing a single cytoplasmic immunoreceptor tyrosine activation motif and a single extracellular C-type lectin recognition domain, and is the predominant receptor in both humans (64, 65) and mice (54) for beta-1,3 glucans (13, 14), the major component of the fungal cell wall. We have shown that mice deficient in Dectin-1 are highly susceptible to lung infection with *A. fumigatus* (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61). We have further reported that a component of susceptibility in Dectin-1 deficiency was lower lung production of IL-17A (61).


