Role of interleukin-18 in experimental infections with Streptococcus pneumoniae

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INTRODUCTION

The Gram-positive bacterium Streptococcus pneumoniae (the pneumococcus) is an important human pathogen worldwide. The most common pneumococcal diseases are pneumonia, bacteremia, meningitis and otitis media. In the USA alone, the pneumococcus is estimated to cause 7,000,000 cases of otitis media, 500,000 cases of pneumonia, 50,000 cases of bacteremia and 3,000 cases of meningitis per year (Obaro, 2000). Additionally, in developing countries, pneumococcal pneumonia is estimated to cause 1,000,000 deaths in children under the age of five (Denny & Loda, 1986). Drawbacks with the current vaccines and the spread of antibiotic resistance pose important difficulties in the prevention and treatment of infection. It is in this setting that continued efforts are made to understand host–pathogen interactions with the ultimate aim of improving therapy.

Several cytokines have been shown to have important functions in host defence against Streptococcus pneumoniae. Among them, IL-18, a pleiotropic cytokine, has been demonstrated to have a key role in infections caused by several bacterial pathogens including group B streptococci (Cusumano et al., 2004), Staphylococcus aureus (Wei et al., 1999), Shigella flexneri (Sanonetti et al., 2000), Yersinia enterocolitica (Bohn et al., 1998), Escherichia coli (Weijer et al., 2002), Salmonella typhimurium (Mastroeni et al., 1999), Pseudomonas aeruginosa (Huang et al., 2002; Schultz et al., 2003) and Legionella pneumophila (Brieland et al., 2000). In the case of Streptococcus pneumoniae, it has been shown that IL-18 is protective in a pneumonia model (Lauw et al., 2002) but detrimental in a meningitis model (Zwijnenburg et al., 2003).

To explore further the influence of IL-18 in pneumococcal infections, we compared the response to infection of wild-type and IL-18 knockout mice. Our data demonstrated that the influence of IL-18 in pneumococcal–host interactions is dependent on the bacterial strain, type of infection and host genetic background. IL-18 therefore has a complex influence in pneumococcal infections.

METHODS

Mice. Mice were housed at the Central Research Facility, University of Glasgow, UK, and all work was carried out with appropriate licensing and approval from the Home Office and University of Glasgow. IL-18 −/− mice were generated as described previously (Wei et al., 1999) and backcrossed on to a BALB/c background over eight generations. Sex- and age-matched (8–13-week-old) homozygous IL-18 −/− and wild-type mice were used in all experiments. Male and female mice gave similar results in the parameters measured. Breeding stock genotypes were confirmed by PCR of genomic DNA as previously described (Jiang et al., 2001). Female MF-1 mice (8–12 weeks old) used for mouse passage of bacterial strains were purchased from Harlan Olac, Bicester, UK.

Bacteria. Strains used were Streptococcus pneumoniae strain D39, serotype 2 (NCTC 7466; Central Public Health Laboratory, London, UK) and the serotype 3 strain ATCC 6303. Bacteria were grown on blood agar base number 2 (Oxoid) with 5% (v/v) defibrinated horse blood (E&O Laboratories) or in brain heart infusion (Oxoid). All incubations were static at 37 °C.
Infection of mice. Prior to use in mice, strains were passaged by intraperitoneal injection in MF-1 mice to maintain virulence as described previously (Alexander et al., 1994), unless otherwise stated. Aliquots of bacteria were stored at −80 °C. When required, aliquots were thawed rapidly, harvested by centrifugation and resuspended in sterile PBS to the required concentration (see doses below). For intranasal infection to induce pneumonia, mice were lightly anaesthetized with halothane (Zeneca Pharmaceuticals) over oxygen using a calibrated vaporizer and 50 μl bacterial suspension (containing the dose indicated) was administered to the nares. Viable counts of the bacterial suspension were determined immediately before and after the challenge to confirm the dose. For intravenous infection, 50 μl bacterial suspension containing 10⁶ c.f.u. was injected into the lateral tail vein. A blood count was taken 1 min after the injection from a separate vein to confirm that the infection was successful. For the colonization model, mice were challenged intranasally with 10⁶ c.f.u. in a 10 μl volume resulting in nasopharyngeal carriage without pneumonia (Kerr et al., 2004).

Bacteriology. In the pneumonia model, mice were sacrificed by cervical dislocation and bacterial viable counts taken from bronchoalveolar lavage fluid (BALF) and lung tissues. For bronchoalveolar lavage, tubing was inserted into the trachea and the lungs lavaged with 2×1 ml PBS. Lungs were then collected into 5 ml PBS and homogenized with an electrical tissue homogenizer (IKA UltraTurrax T25; Janke and Kunkel). Serial 10-fold dilutions of both lavage fluid and homogenized lung tissue were plated out and viable counts determined. Counts were pooled to give the total lung count. Following intranasal challenge, mice were tail bled at pre-determined time points and viable counts determined. For the colonization model, mice were sacrificed by cervical dislocation and bacterial viable counts taken from nasal washings with 2 ml PBS.

Cytokine analysis. Aliquots of BALF were snap frozen in liquid nitrogen and stored at −80 °C until analysis for cytokines. Interferon (IFN)-γ was measured by ELISA using paired antibodies from Pharmingen (catalogue nos 554431 and 554410; detection limit ∼15 pg ml⁻¹).

Statistics. Bacterial counts and IFN-γ levels were compared by Mann–Whitney U test analysis, where P < 0.05 was considered significant.

RESULTS

Influence of IL-18 on pneumococcal pneumonia

To assess the role of IL-18 in pneumococcal pneumonia, bacterial loads in the lungs of wild-type and IL-18 knockout BALB/c mice were compared after intranasal challenge with 10⁶ c.f.u. serotype 2 strain D39. At 24 h, IL-18 knockout mice showed enhanced clearance of bacteria compared with their wild-type counterparts (Fig. 1a). This difference was transient, as bacterial counts in the lung were similar in the two mouse strains at 12 and 48 h post-infection (Fig. 1a). In this model, therefore, IL-18 appeared to reduce bacterial clearance slightly at 24 h. A major activity of IL-18 is the induction of IFN-γ. As previous work has demonstrated a detrimental role for IFN-γ in murine pneumococcal pneumonia, we compared the levels of this cytokine in BALF between wild-type and IL-18 knockout mice (Rijneveld et al., 2002). At 24 h post-infection, there was a significant reduction in IFN-γ levels in the IL-18 knockout mice compared with wild-type (mean ± SEM for wild-type mice of 259 ± 45 pg ml⁻¹ compared with 116 ± 8 pg ml⁻¹ for knockout mice, P < 0.05).

A bacterial-strain-dependent role for IL-18 in pneumococcal pneumonia

To determine whether the influence of IL-18 on pneumococcal pneumonia shown for D39 was specific to the strain used, we repeated the infection using the serotype 3 strain ATCC 6303. At the time points examined, no significant difference was seen between the two mouse strains (Fig. 1b). This demonstrated that the effect of IL-18 on pneumococcal pneumonia was influenced by the bacterial strain used. When BALF IFN-γ was measured, there was no significant difference between the two strains, although there was a strong trend for higher levels in the wild-type mice (data not shown).

Role of IL-18 in other pneumococcus–host interactions

To examine the role of IL-18 in other pneumococcal infection types, BALB/c wild-type and IL-18 knockout mice were compared following intravenous infection to represent bacteraemia and in a nasopharyngeal colonization model. Following intravenous infection, no difference was seen in blood bacterial counts between the two mouse strains at the time points examined (6, 12 and 24 h; data not shown). During nasopharyngeal colonization, IL-18 knockout mice showed diminished clearance of D39 at day 7 (Fig. 2). This effect was transient as counts at day 3 and 10 were not significantly different between the strains, although at day 10 IL-18 knockout mice showed a trend towards higher counts. IFN-γ levels were below the detection limit of 15 pg ml⁻¹ in the nasopharyngeal washings.
In these models, therefore, IL-18 did not appear to play a significant role in bacteraemia infection, but contributed towards controlling levels of colonization in the nasopharynx. The influence of IL-18 in pneumococcal infection is therefore complex and varies with the infection model.

Mouse strain influence on the role of IL-18 in pneumococcal pneumonia

Data from our pneumonia infections with the strains D39 and ATCC 6303 are in disagreement with the work of Lauw et al. (2002). Using strain ATCC 6303, they demonstrated a protective influence of IL-18 in pneumococcal pneumonia, whereby IL-18 knockout mice showed enhanced bacterial counts in the lung compared with wild-type mice. In addition to employing a different mouse strain (C57/Bl6) to us, their work also administered a smaller dose (10⁵ c.f.u.) and used bacteria that were not first mouse passaged. To resolve the discrepancy between these results, we repeated our pneumonia infection using unpassaged ATCC 6303 at 10⁵ c.f.u. per mouse. When lung bacterial counts were compared between mouse strains at 48 h post-infection, no difference was evident (data not shown). Forty-eight hours was chosen because Lauw et al. (2002) showed the greatest difference in bacterial counts between the mouse strains at this time point.

DISCUSSION

To investigate the role of IL-18 in pneumococcal pneumonia, we compared bacterial lung counts between wild-type and IL-18 knockout BALB/c mice following infection with 10⁶ c.f.u. serotype 2 strain D39. BALB/c mice in this infection model are a comparatively resistant strain and have been studied in attempts to understand genetic resistance to the pneumococcus (Gingles et al., 2001; Kerr et al., 2002). Our data suggested that IL-18 does not contribute to the inherent resistance of BALB/c mice in this model. Indeed, IL-18 in this model was responsible for a transient decrease in bacterial clearance and may therefore be classed as detrimental. The phenotype appears only minor, as bacterial counts were the same in both genotypes later in infection. BALB/c mice are also relatively resistant to intravenous infection with D39 (Kerr et al., 2002). Our data demonstrated that IL-18 is not essential for this resistance. The resistance of BALB/c mice to D39 intranasal and intravenous infection correlates with abundant and early production of TNF-α after infection (Kerr et al., 2002). The dominant influence of this cytokine in BALB/c mice following pneumococcal infection may explain the relatively minor phenotype seen in IL-18 knockout mice.

The decreased clearance of D39 in the wild-type mice during pneumonia correlated with increased IFN-γ production compared with IL-18 knockout mice. This cytokine has been shown previously to be detrimental in a model of pneumococcal pneumonia (Rijneveld et al., 2002) and the higher levels seen here in wild-type mice may have contributed to the reduced bacterial clearance relative to the IL-18 knockout mice.

When the pneumonia infection was repeated using the serotype 3 strain ATCC 6303, no difference in lung bacterial counts was seen between the two mouse strains. This demonstrated that the influence of IL-18 in pneumococcal pneumonia is determined by the infecting bacterial strain. The reason is unclear, but it has been documented that different pneumococcal strains can induce different cytokine responses and this may be a contributing factor whereby one strain may induce higher or lower levels of beneficial or detrimental cytokines (Mohler et al., 2003).

We also demonstrated that the role of IL-18 in pneumococcal infection varies with different types of infection. Whereas in pneumonia it impaired bacterial clearance, the opposite was seen in a model of nasopharyngeal colonization, while, in contrast, no effect was seen in a bacteraemia model. This expands previous data showing a protective role in pneumococcal pneumonia (Lauw et al., 2002) but a detrimental one in a meningitis model (Zwijnenburg et al., 2003). Similarly, IL-18 is protective in a mouse model of P. aeruginosa keratitis (Huang et al., 2002) but impaired bacterial clearance in a pneumonia model with the same organism (Schultz et al., 2003). Furthermore, IL-18 administration had opposing effects on E. coli infections in burn-injured mice depending on the severity of the burns (Kinoshita et al., 2004).

In addition to the influence of bacterial strain and infection type on the role of IL-18 in pneumococcal infection, it appears that host genetic background is also important. Previously, Lauw et al. (2002) demonstrated that IL-18 knockout C57/Bl6 mice had increased bacterial lung counts compared with wild-type mice following pneumococcal pneumonia with strain ATCC 6303. When we repeated this infection using BALB/c mice, no difference was seen between the wild-type and IL-18 knockout mice with regard to bacterial lung counts. It therefore seems that IL-18 influences pneumococcal pneumonia differently depending on the host genetic background. A similar effect has been described for the role of IL-18 in response to Leishmania major infection in mice (Wei et al., 2004).

Taken together, we have demonstrated that IL-18 exerts...
differing effects on pneumococcal infection depending on the infecting bacterial strain, the infection type and the host genetic background. These data exemplify the complexity of IL-18 in the immune response to *Streptococcus pneumoniae*.

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**REFERENCES**


