The Role of Cytokines in the Pathogenesis of Pneumonia

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Introduction

Bacterial pneumonia is a major cause of morbidity and mortality worldwide, despite the armamentarium of antibacterial agents, advanced diagnostic technologies and sophisticated, sometimes aggressive treatments used in intensive care facilities. In developing countries community-acquired pneumonia causes ten times as many deaths as all infectious diseases together [1]. Pneumonia is the second most frequent cause of hospital-acquired infection in the United States [2].

Adult patients on the intensive care unit receiving mechanical ventilation suffer the highest occurrence rate and mortality is particulary high in these critically ill patients. Treatment of bacterial pneumonia is hampered by the increased antibiotic-resistance of pathogens [3]. Immune host defense continues to play an important role in the outcome of bacterial pneumonia, and modulation of the inflammatory response has been considered as an adjunctive treatment strategy.

Normally, bacteria are prevented from reaching the alveoli by several defense mechanisms located along the upper airway. Bacteria reaching the alveoli are usually phagocytosed and killed by alveolar macrophages. When these normal protective mechanisms are overwhelmed, several complex defense systems are triggered. The invasion of pathogens produces a vigorous inflammatory response, including the recruitment of neutrophils. Neutrophils exert microbicidal effects involving several oxidative and enzymatic processes [4, 5]. In addition, complement products can promote the killing of bacteria by neutrophils and macrophages [6, 7].

Multiple lines of scientific evidence have demonstrated the crucial role of a complex network of cytokines in the initiation and maintenance of inflammation during bacterial infection. Much of our understanding of the role of cytokines is based on results from studies on overwhelming immune activation in the absence of a localized source, such as is induced by intravenous administration of bacterial products. Although excessive pro-inflammatory cytokine production during severe infection may have deleterious effects, more recent studies support the beneficial role of pro-inflammatory cytokines in local host defense.

Cytokines in Systemic Infections and Experimental Endotoxemia

Cytokines are small glycoproteins (6–30 kD) that can be produced by, and have effects on a large variety of cell types. The affinity of cytokines for their receptors is

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very high, enabling effects at picomolar concentrations. Mononuclear activation by bacteria or bacterial products, like lipopolysaccharide (LPS), leads to the production of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6 and IL-8 [8, 9], as well as the anti-inflammatory cytokine IL-10 [8]. TNF, IL-10 and interferon-γ (IFN-γ) are also produced by T cells and B cells after anti-genic stimulation. The pro- and anti-inflammatory cytokines interact in a complex network, in which they can influence each other's production and/or activity.

Much of our knowledge about the function of the cytokine network is derived from studies on systemic infections. Severe bacterial infection can result in profound physiologic changes including hypotension, fever, tissue necrosis, multiple organ dysfunction and finally death. In Gram-negative infections, these changes are due, at least in part, to endotoxin, the LPS component of the bacterial cell wall. Injection of LPS into animals produces changes that are typical of the sepsis syndrome. During such overwhelming immune activation, TNF and IL-1 are considered to be important mediators of systemic inflammatory responses [9, 10]. TNF and IL-1β have pleiotropic effects, including activation of neutrophils and the extrinsic pathway of coagulation, and stimulation of neutrophilic adherence to endothelial cells. It is clear that these cytokines play an orchestrating role in the generation of inflammation after an intravenous bolus of LPS in healthy humans. TNF is the first cytokine to appear [8] with peak plasma levels reached within 90 minutes following LPS administration. After the release of TNF, serum concentrations of other cytokines such as IL-6 and IL-8 increase. TNF is responsible for the release of these cytokines, since neutralization of TNF activity prevents the appearance of these secondary cytokines during endotoxemia [11]. Elimination of IL-6 activity does not affect the induction of other cytokines [12], indicating that IL-6 is a more distal cytokine in the LPS-induced cytokine cascade.

IL-10 is produced under different conditions of immune activation by different cell types, including T-cells, B-cells and monocytes [13]. In sepsis and after LPS administration, circulating levels of IL-10 are elevated. IL-10 production is also enhanced in various models of infection, like experimental endotoxemia, staphylococcal enterotoxin B-induced lethal shock, and septic peritonitis. IL-10 can inhibit the LPS-stimulated production of pro-inflammatory cytokines *in vitro* and *in vivo*. IL-10 plays a protective role in models of overzealous inflammation, since administration of IL-10 reduces LPS-mortality in animals, and neutralization of IL-10 results in increased lethality in LPS-challenged mice [13].

An important problem in interpreting models of Gram-negative sepsis and experimental endotoxemia is that they do not provide insight into the local production and interactions of cytokines at the site of an infection, e.g., within the lung during pneumonia. In recent years, several studies have provided information about local cytokine production during pneumonia, in human as well as in animal models.

The Cytokine Cascade in Pulmonary Infection

In local infection, like pneumonia, the initiation, maintenance and resolution of inflammation are considered to be dependent upon the expression of the complex network of pro-inflammatory and anti-inflammatory cytokines. In the last decade

numerous studies have been performed to determine systemic and local levels of pro-inflammatory and anti-inflammatory cytokines in patients with pneumonia. In a study comprising 64 patients with community-acquired pneumonia with a well-defined etiology, Kragsbjerg et al. [14] found high concentrations of TNF, IL-6, IL-8 and IFN-γ in sera taken on admission to the hospital. Serum TNF was elevated in most patients, irrespective of the etiology, while serum IL-6 was highest in patients with pneumococcal pneumonia, and patients with pneumonia due to *Legionella* species. None of the patients with pneumonia due to pathogens like *Haemophilus influenzae*, *Moraxella catarrhalis* and *Chlamydia pneumoniae*, had a serum IL-6 level above 500 pg/ml. On the other hand, serum IFN-γ concentrations were elevated in most of the patients with viral or intracellular bacterial disease, the highest values being detected in patients with pneumonia due to *Legionella* species and *Chlamydia pneumoniae*. Elevated IFN-γ levels were seen in a minority of patients with pneumococcal pneumonia. In the second study by Kragsbjerg et al. [15], involving 63 patients with acute respiratory tract infection, serum IL-8 levels on admission were significantly higher among patients with bacteremic pneumococcal pneumonia, than in patients with *Chlamydia* pneumonia, *Legionella* pneumonia or influenza A virus infection. Other studies have confirmed the presence of elevated serum concentrations of TNF, IL-1β and IL-6 in patients with community-acquired pneumonia [16, 17].

Investigations examining cytokine concentrations in pleural and bronchoalveolar lavage (BAL) fluid have suggested that during pneumonia, cytokines are produced locally at the site of the infection. In a study of 102 patients with pleural effusion of different etiologies, Silva-Mejias et al. [18] found that IL-1 levels were elevated in pleural fluids of all 14 patients with empyema. In pleural effusions of other etiologies (transudates, parapneumonic, tuberculous, neoplastic and miscellanous), only 3 patients, 2 with parapneumonic pleural effusion and 1 with tuberculous pleural effusion, had elevated IL-1β levels in the pleural fluid [18]. Broaddus et al. [19] found higher IL-8 levels in pleural empyema fluids, compared with other types of effusion. IL-8 levels correlated with neutrophil counts in pleural fluid, and pleural fluid neutrophil chemotactic activity [19]. Interestingly an anti-IL-8 antibody decreased the neutrophil chemotactic activity of pleural fluid, suggesting that IL-8 plays an important role in the recruitment of neutrophils in empyema.

Dehoux et al. [20] and Boutten et al. [21] studied local cytokine production by measuring cytokine levels in BAL fluid from 15 patients admitted for unilateral community-acquired pneumonia. Cytokine concentrations in BAL fluid from the infected lung were compared with those in BAL fluid from the contralateral, non-involved lung and in BAL fluid from healthy individuals. In addition, cytokine levels were determined in sera, to enable comparison between local and systemic cytokine concentrations. It was found that TNF, IL-1β, IL-6 and IL-8 concentrations were significantly higher in the infected lung than in the non-involved lung or serum, indicating that the cytokine response during unilateral pneumonia is compartmentalized and limited to the site of infection (Fig. 1). Schutte et al. [22] compared cytokine concentrations in BAL fluid and serum of patients with acute respiratory failure due to cardiogenic pulmonary edema (6 patients), adult respiratory distress syndrome (ARDS, 12 patients), primary severe pneumonia (38 patients) or a combination (pneumonia and ARDS, 18 patients). In all patients with ARDS and/or pneu-

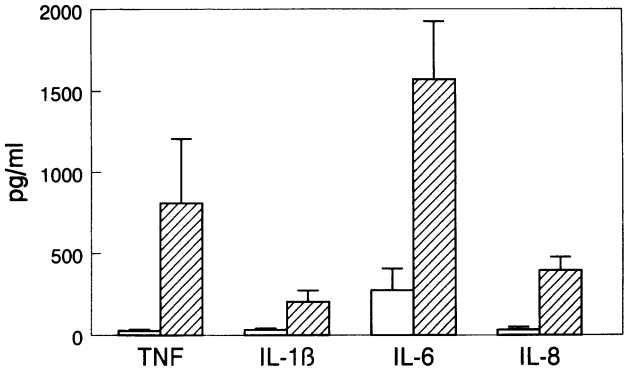

Fig. 1. Compartmentalized cytokine production during pneumonia. Mean (± SE) cytokine concentrations in bronchoalveolar lavage (BAL) fluid obtained from patients with unilateral community acquired pneumonia. Open bars represent cytokine levels in BAL fluid from the non-involved lung, hatched bars represent cytokine levels in BAL fluid from the infected lung. (Adapted from [20] and [21] with permission)

monia elevated BAL fluid levels of IL-6 and IL-8 were detected. Serum IL-6 levels were also elevated in these patients, whereas IL-8 levels were increased inconsistently.

In addition to cytokine measurements in BAL fluid and serum, Dehoux et al. [20] also examined cytokine production by alveolar and peripheral macrophages *ex vivo*. Alveolar macrophages recovered from the infected lung spontaneously released more TNF, IL-1β and IL-6 into cell culture supernatants than macrophages evacuated from the non-involved lung. The spontaneous release of these cytokines by macrophages from the non-involved lung was comparable to those from lungs of healthy controls. After stimulation with LPS *ex vivo*, cytokine concentrations reached in cell culture supernatants were similar when cells from the involved lung and non-involved lung of the patients were compared, but much lower than those measured in control subjects. This hypo-responsiveness to *in vitro* LPS stimulation was not observed in the cultures of peripheral monocytes. These data are in line with reports on LPS hyporesponsiveness of mononuclear cells from peripheral blood of patients with severe systemic infections [23], and support the existence of a compartmentalized inflammatory response during pneumonia. Huang et al. [24] measured cytokine release from alveolar macrophages obtained from patients with acquired immunodeficiency syndrome (AIDS) and *Pneumocystis carinii* pneumonia who received or did not receive corticosteroids. LPS stimulation resulted in significantly less TNF and IL-1β release from alveolar macrophages of patients receiving corticosteroids, indicating that local cytokine production can be down-regulated by this steroid hormone.

At present no studies have been published concerning the local production of anti-inflammatory cytokines in pneumonia in humans.

Studies on Cytokine Production in Animal Models of Pneumonia

Recently, several studies in animals with experimental respiratory tract infections have confirmed the suggestion that in pneumonia, like in systemic infections, inflammation is orchestrated by pro-inflammatory and anti-inflammatory cytokines (Table 1). In murine pneumococcal pneumonia, induced by intranasal instillation of 10^6 colony forming units (CFU) of *Streptococcus pneumoniae*, increased levels of TNF were found from day 1 in serum and from day 3 in lungs, concomitant with an increase in bacterial counts in lungs [25]. The experimental infection was worsened by intravenous administration of an anti-TNF antibody, since this treatment enhanced bacterial proliferation in the blood, compared with mice treated with normal rabbit serum. These results suggest that TNF prevents the onset of bacteremia, and plays a protective role in experimental pneumococcal pneumonia. Indeed mortality was significantly higher in anti-TNF-treated mice. Similar results were obtained in another murine pneumococcal pneumonia investigation [26]. Intranasal inoculation of 10^6 CFU of *Streptococcus pneumoniae* resulted in a sustained release of TNF in lung homogenates reaching a plateau between 12 and 72 hours. Intraperitoneal treatment with a neutralizing anti-TNF antibody two hours before inoculation strongly reduced TNF levels in lungs, while IL-1β levels were only modestly affected and IL-6, IL-10 and IFN-γ concentrations were unchanged. Mice treated with anti-TNF had four fold more *Streptococcus pneumoniae* CFU isolated from lungs than control mice 40 hours after inoculation, and died significantly earlier from pneumococcal pneumonia (Fig. 2). Compelling evidence in support of TNF as an important mediator in bacterial pneumonia with other than Gram-positive pathogens is derived from models of Gram-negative bacterial respiratory tract infections. In murine pneumonia induced by an intratracheal challenge with 10^2 CFU of *Klebsiella pneumoniae*, anti-TNF treatment was associated with a markedly decreased survival, increased bacterial growth in lung homogenates and blood, and a significant reduction in BAL neutrophils at 48 hours after inoculation [27]. Anti-TNF had the same effect on growth of *Legionella pneumophila* in lungs in a pneumonia model induced by intratracheal inoculation of 10^6 CFU of this microorganism [28]. In mice with pneumonia caused by *Pneumocystis carinii* anti-TNF almost completely prevented the clearance of *Pneumocystis* from the lungs [29]. In addition, treatment of granulocytopenic mice with low doses of TNF and/or IL-1β significantly diminished mortality and enhanced pulmonary clearance of *Pseudomonas*

Table 1. Role of endogenous cytokines, produced within the pulmonary compartment, during pneumonia in mice

Protective	Detrimental
TNF	IL-10
IL-6	
IFN-γ	
IL-12	
MIP-2	

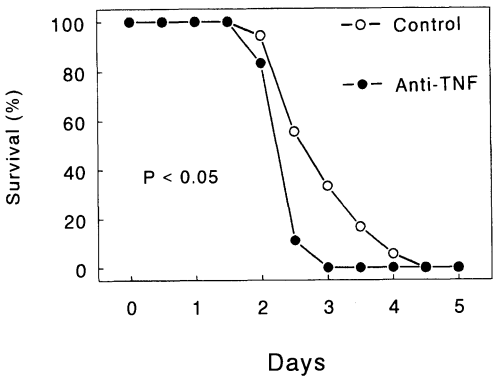

Fig. 2. Anti-TNF impairs host defense during murine pneumococcal pneumonia. Mice were intranasally inoculated with 10^6 CFU *Streptococcus pneumoniae* after intraperitoneal administration of a neutralizing anti-TNF mAb (1 mg) or an irrelevant control mAb at t = −2 hours. (Adapted from [26] with permission)

aeruginosa during severe pneumonia [30]. Hence, endogenously produced TNF is important for host defense during experimental pneumonia induced by different microorganisms.

Macrophage inflammatory protein-2 (MIP-2) is an important mediator of inflammation, inducing potent chemotaxis of neutrophils. Greenberger et al. [31] found time-dependent expression of MIP-2 mRNA and protein within the lung during murine *Klebsiella* pneumonia. Treatment of mice with an anti-MIP-2 antibody caused a 60% decrease in lung neutrophil influx, a significant increase of *Klebsiella pneumoniae* CFU in lungs and liver homogenates, and a decrease in early survival. These results indicate the important role of MIP-2 in neutrophil influx and bacterial clearance during pneumonia.

Evidence for the importance of IL-6 in host defense is obtained from a study on pneumococcal pneumonia in IL-6 deficient mice [32]. Intranasal inoculation of 10^6 CFU of *Streptococcus pneumoniae*, resulted in sustained expression of IL-6 mRNA and protein in lungs. Higher levels of the pro-inflammatory cytokines TNF, IL-1β and IFN-γ and the anti-inflammatory cytokine IL-10 were found in the lungs of IL-6 deficient mice with pneumonia. IL-6 deficient mice had more CFU of *Streptococcus pneumoniae* in their lungs, and died significantly earlier than normal mice. Hence, IL-6 down-regulates the activation of the cytokine network within the lung during pneumonia and contributes to host defense.

IFN-γ is another pro-inflammatory cytokine that seems to be important for resistance against Gram-positive respiratory tract infections. IFN-γ production is enhanced during murine pneumococcal pneumonia [26, 33], and IFN-γ deficient mice show a markedly increased susceptibility to infection with *Streptococcus pneumoniae* [33]. However, anti-IFN-γ treatment had no detectable effect on the clearance of *Pneumocystis carinii* from lungs [29].

The importance of another pro-inflammatory cytokine, IL-12, was recently demonstrated in mice suffering from pneumonia with *Klebsiella pneumoniae* [34]. Intratracheal challenge with 10^2 CFU *Klebsiella pneumoniae* resulted in a time dependent expression of IL-12 mRNA and protein in the lung. Passive immunization with a polyclonal anti-IL-12 antibody at the time of infection with *Klebsiella pneumoniae* resulted in a marked increase in bacterial counts in lung homogenates 48 hours after inoculation, as compared with mice receiving control serum. More-

over, treatment with anti-IL-12 decreased survival. Overexpression of IL-12 within the lung by intratracheal administration of an adenoviral vector containing the human cytomegalovirus promoter and cDNA coding for IL-12, resulted in 45% long term survival in *Klebsiella* pneumonia, while none of the mice receiving control adenovirus survived. In the same experiment [34], passive immunization against TNF or IFN-γ led to a failure of IL-12 overexpression to protect mice, indicating that IL-12 does not act alone in the defense against invading microorganisms.

Considerable evidence exists that the anti-inflammatory cytokine IL-10 plays a detrimental role in the clearance of bacteria during pulmonary infections [35, 36]. Intratracheal installation of 10^3 CFU *Klebsiella pneumoniae* was associated with increased IL-10 mRNA and protein levels in lung homogenates [35]. Passive immunization of mice with a polyclonal anti-IL-10 antibody resulted in significantly higher levels of TNF in lung homogenates, than in mice that received pre-immune serum. Importantly, *Klebsiella pneumoniae* CFU in lungs of mice treated with anti-IL-10 were approximately eight fold less, than in mice treated with pre-immune serum, while *Klebsiella pneumoniae* CFU in plasma were over a hundred fold less. Although intratracheal instillation of *Klebsiella pneumoniae* resulted in 100% lethality in all mice, survival was significantly longer in anti-IL-10 treated mice. Similar results were obtained in studies with experimental pneumonia with Gram-positive bacteria [36]. Intranasal administration of *Streptococcus pneumoniae* resulted in a marked increase in IL-10 in lung homogenates, the highest lung IL-10 levels being measured at 72 hours after inoculation with pneumococci. Treatment of mice with recombinant IL-10 resulted in a decrease in lung TNF levels, while administration of an anti-IL-10 antibody resulted in a three and a half fold rise in lung TNF and IFN-γ levels. In animals treated with anti-IL-10, bacterial counts from lung and blood were lower and survival was significantly increased (Fig. 3). Together with the results from the study of Greenberger et al. [35], these results indicate that during pneumonia, IL-10 attenuates the pro-inflammatory cytokine response within the lungs, hampers effective clearance of infection and shortens survival.

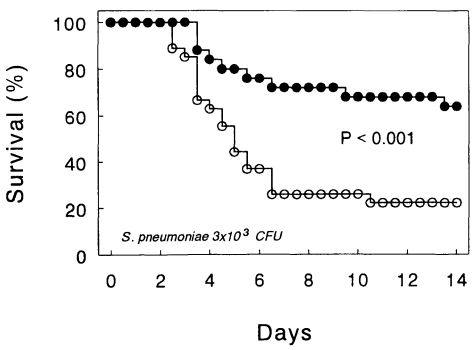

Fig. 3. Anti-IL-10 protects mice against lethality during pneumococcal pneumonia. Mice were intranasally inoculated with 3×10^3 CFU *Streptococcus pneumoniae* after intraperitoneal administration of a neutralizing anti-IL-10 mAb (2 mg; closed circles) or an irrelevant control mAb (open circles) at t = −2 hours. (Adapted from [36] with permission)

Conclusion

As in systemic infection, inflammation during pneumonia is orchestrated by locally produced pro-inflammatory and anti-inflammatory cytokines. There are however some important differences between the role of cytokines during localized infection and during fulminant systemic infection. Whereas excessive production of pro-inflammatory cytokines at the systemic level causes organ failure and death in animal models of fulminant sepsis, the local production of these cytokines importantly contributes to host defense against pneumonia. Conversely, while the anti-inflammatory cytokine IL-10 is protective in models of overzealous immune activation, it impairs host defense during pneumonia. These findings emphasize the importance of clinically relevant animal models to fully understand the role of cytokines in the pathogenesis of bacterial infection. Local modulation of the cytokine network may serve as an important addition to antibiotic therapy, especially when faced with multi-drug resistant organisms and/or immunocompromised hosts.

References

1. British Thoracic Society Research Committee (1987) Community-acquired pneumonia in adults in British hospitals in 1982–1983: A survey of aetiology, mortality, prognostic factors and outcome. Q J Med 62:195–220
2. Horan T, Culver D, Jarvis W (1988) Pathogens causing nosocomial infections. Antimicrob Newsletter 5:65–67
3. Davies J (1994) Inactivation of antibiotics and the dissemination of resistance genes. Science 264:375–382
4. Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320:365–375
5. Tsai WC, Strieter RM, Zisman DA, et al (1997) Nitric Oxide is required for effective innate immunity against *Klebsiella pneumoniae*. Infect Immun 65:1870–1875
6. Hopken UE, Lu B, Gerard NP, Gerard C (1996) The C5a chemoattractant receptor mediates mucosal defense to infection. Nature 383:86–89
7. Winkelstein JA (1981) The role of complement in the host's defense against *Streptococcus pneumoniae*. Rev Infect Dis 3:289–297
8. Pajkrt D, van der Poll T, van Deventer SJH (1997) Inflammatory responses during human endotoxemia. In: Vincent JL (ed) 1997 Yearbook of intensive care and emergency medicine. Springer-Verlag, Berlin, Heidelberg, pp 14–30
9. Van der Poll T, Lowry SF (1995) Tumor necrosis factor in sepsis: Mediator of multiple organ failure or essential part of host defense? Shock 3:1–12
10. Dinarello CA (1996) Biological basis for interleukin 1 in disease. Blood 87:2095–2147
11. Suffredini AF, Reda D, Banks SM, Tropea M, Agosti JM, Miller R (1995) Effects of recombinant dimeric TNF receptor on human inflammatory responses following intravenous endotoxin administration. J Immunol 155:5038–5045
12. Van der Poll T, Levi M, Hack CE, et al (1994) Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J Exp Med 179:1253–1259
13. Moore KW, O'Garra A, de Waal Malefyt R, Vierra P, Mosmann TR (1993) Interleukin-10. Annu Rev Immunol 11:165–190
14. Kragsbjerg P, Vekerfors T, Holmberg H (1993) Serum levels of interleukin 6, tumor necrosis factor alpha, interferon gamma and C-reactive protein in adults with community-acquired pneumonia. Serodiagn Immunoth Infect Disease 5:156–160
15. Kragsbjerg P, Jones I, Vikerfors T, Holmberg H (1995) Diagnostic value of blood cytokine concentrations in acute pneumonia. Thorax 50:1253–1257
16. Puren AJ, Feldman C, Savage N, Becker PJ, Smith C (1995) Patterns of cytokine expression in community-acquired pneumonia. Chest 107:1342–1349

17. Moussa K, Michie HJ, Cree IA, et al (1994) Phagocyte function and cytokine production in community-acquired pneumonia. Thorax 49:107–111
18. Silva-Mejias C, Gamboa-Antinolo F, Lopez-Cortez LF, Cruz-Ruiz M, Pachon J (1995) Interleukin 1 beta in pleural fluids of different etiologies. Its role as inflammatory mediator in empyema. Chest 108:942–945
19. Broaddus VC, Hebert CA, Vitangcol RV, Hoeffel JM, Bernstein MS, Bylan AM (1992) Interleukin-8 is a major neutrophil chemotactic factor in pleural liquid of patients with empyema. Am Rev Respir Dis 146:825–830
20. Dehoux MS, Boutten A, Ostinelli J, et al (1994) Compartmentalized cytokine production within human lung in unilateral pneumonia. Am J Resp Crit Care Med 150:710–716
21. Boutten A, Dehoux MS, Seta N, et al (1996) Compartmentalized IL-8 and elastase release within the human lung in unilateral pneumonia. Am J Respir Crit Care Med 153:336–342
22. Schutte H, Lohmeyer J, Rosseau S, et al (1996) Bronchoalveolar and systemic cytokine profiles in patients with ARDS, severe pneumonia and cardiogenic pulmonary oedema. Eur Respir J 9:1858–1867
23. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM (1991) Dysregulation of in vitro cytokine production by monocytes during sepsis. 88:1747–1754
24. Huang ZB, Eden E (1993) Effect of corticosteroids on IL-1β and TNF alpha release by alveolar macrophages from patients with AIDS and Pneumocystis carinii pneumonia. Chest 104:751–755
25. Takashima K, Tateda K, Matsumoto T, Iizawa Y, Nakao M, Yamaguchi K (1997) Role of tumor necrosis factor alpha in the pathogenesis of pneumococcal pneumonia in mice. Infect Immun 65:257–260
26. Van der Poll T, Keogh CV, Buurman WA, Lowry SF (1997) Passive immunization against tumor necrosis factor alpha impairs host defense during pneumococcal pneumonia. Am J Resp Crit Care Med 155:603–608
27. Laichalk LL, Kunkel SL, Strieter RM, Danforth JM, Bailie MB, Standiford TJ (1996) Tumor necrosis factor mediates lung antibacterial host defense in murine *Klebsiella* pneumonia. Infect Immun 64:5211–5218
28. Brieland JK, Remick DG, Freeman PJ, Hurley MC, Fontane JC, Engleberg NC (1995) In vivo regulation of replicative *Legionella pneumophila* lung infection by endogenous tumor necrosis factor alpha and nitric oxide. Infect Immun 63:3253–3258
29. Chen W, Havell EA, Harmsen AG (1992) Importance of endogenous tumor necrosis factor alpha and interferon gamma in host resistence against *Pneumocystis carinii* infection. Infect Immun 60:1279–1284
30. Amura C, Fontan PA, Sanjuan N, Sordelli DO (1994) The effect of treatment with interleukin-1 and tumor necrosis factor on *Pseudomonas aeruginosa* lung infection in a granulocytopenic mouse model. Clin Immunol Immunopath 2:261–266
31. Greenberger MJ, Strieter RM, Kunkel SL, et al (1996) Neutralization of macrophage inflammatory protein-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella* pneumonia. J Infect Dis 173:159–165
32. Van der Poll T, Keogh CV, Guirao X, Buurman WA, Kopf M, Lowry SF (1997) Interleukin-6 gene deficient mice show impaired defense against Streptococcal pneumonia. J Infect Dis 176: 439–444
33. Rubins JB, Pomeroy C (1997) Role of gamma interferon in the pathogenesis of bacteremic pneumococcal pneumonia. Infect Immun 65:2975–2977
34. Greenberger MJ, Kunkel SL, Strieter RM, et al (1996) IL-12 gene therapy protects mice in lethal *Klebsiella* pneumonia. J Immunol 157:3006–3012
35. Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Goodman RE, Standiford TJ (1995) Neutralizing of IL-10 increases survival in a murine model of *Klebsiella* pneumonia. J Immunol 155:722–729
36. Van der Poll T, Marchant A, Keogh CV, Goldman M, Lowry SF (1996) Interleukin-10 impairs host defense in murine pneumococcal pneumonia. J Infect Dis 174:994–1000