Molecular and Cellular Biochemistry **217:** 107–112, 2001.

Levels of IL-8 and myeloperoxidase in the lungs of pneumonia patients

H. Abul,[1] A. Abul,[2] I. Khan,[3] T.C. Mathew,[4] A. Ayed[5] and E. Al-Athary[1]

Departments of [1]Pharmacology, [2]Medicine, [3]Biochemistry, [5]Surgery, Faculty of Medicine; [4]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences and Nursing, Kuwait University Health Sciences Center, Kuwait

Received 23 June 2000; accepted 2 November 2000

Abstract

Interleukin-8 (IL-8) is considered as the major polymorphonuclear neutrophils (PMNs) chemoattractant cytokine in lung diseases such as asthma and adult respiratory distress syndrome (ARDS). However, controversial results were obtained regarding the involvement of IL-8 in the pathogenesis of pneumonia. This study examines the role of IL-8 in the recruitment and activation of PMNs in the lung of pneumonia patients. The interesting aspect of this study is that it is a site- specific analysis of the infected and uninfected lungs of the same patient. The level of IL-8 mRNA, protein and myeloperoxidase present in the cells of the bronchioalveolar lavages (BALs) taken from the areas of known pneumonic consolidations on chest X-ray (infected lung) are compared with the BALs obtained from areas of no obvious infiltrate (non-infected lung). The results obtained from the infected and non-infected lungs of pneumonic patients were further compared with that of a control group of non-smoking patients. The level of IL-8 mRNA and protein were determined by RT-PCR and ELISA respectively. There was a significant increase in the level of IL-8 mRNA in the infected lung as compared to its level in the non-infected lung ($p < 0.001$). In correlation with the increase in mRNA, IL-8 protein concentrations in BAL fluids from the infected lung were 6 fold higher than those taken from the non-infected lung ($p < 0.0001$). This pattern was also consistent with MPO activity in the BALs (4.5 fold more MPO activity in the infected lung as compared to that of the non-infected lung), indicating that IL-8 is directly implicated in neutrophil accumulation that follows acute respiratory infection. The results of the present study, therefore, indicate the involvement of IL-8 in the pathogenesis of pneumonia. (Mol Cell Biochem **217**: 107–112, 2001)

Key words: interleukin-8, bronchioalveolar lavage, polymerase chain reaction, IL-8 mRNA, myeloperoxidase, pneumonia

Introduction

Chemokines constitute a large family of regulatory cytokines that play a central role in immunological processes. The accumulation and appearance of polymorphonuclear neutrophils (PMNs) in the tissue may be considered as an initial marker of acute inflammatory reaction [1]. Neutrophils participate in the host response to a number of infectious and non-infectious diseases and in leukocyte migration [2–6]. They contain cytoplasmic granules that function in storage of bioactive neuromolecules (specific or secondary granules) or in fusion with phagosomes (azurophilic or primary granules). The azurophilic granules contain a variety of enzymes including myeloperoxidase, muraminidase, cathepsin A, D, E, G, 5′-nucleotidase, β-galactosidase, elastase, collagenase, azurocidin and the defensins HNP-1, HNP-2, and HNP-3, arylsulfatase, α-mannosidase, *N*-acetyl-β-glucosaminidase, β-glucuronidase, acid β-glycerophosphatase and cationic peptides. The specific granules on the other hand contain vitamin-B_{12}-binding protein, neutral proteases, lactoferrin, alkaline phosphatase, lysozyme, and probably collagenase [7]. Although the mechanisms that regulate the release of substances from both the granules are almost the same, there are certain specific stimuli such as IL-8 and zymosan that induce the release of substances from secondary granules [8]. Thus IL-8 functions as a potent chemotacting as well as degranulating agent.

Recently it has been shown that depletion of neutrophils using anti-rat neutrophil antiserum reduced subsequent development of chronic delayed type hypersensitivity reactions

Address for offprints: H. Abul, Department of Pharmacology, Faculty of Medicine, P.O. Box 24923, 13110 Safat, Kuwait

[9]. This study clearly demonstrates the importance of neutrophil derived factors for monocyte and lymphocyte mobilization. Furthermore, it is shown that neutrophils produce a number of low molecular weight factors such as leukotriene B4 (L BT4) that attract more neutrophils and monocytes to the inflammatory site [10]. T-lymphocytes have also been shown to migrate in response to IL-8 both *in vivo* and *in vitro* [11, 12].

There is a clear involvement of IL-8 in the pathophysiology of various respiratory diseases [13–17]. In asthma, airway inflammation with eosinophils, lymphocytes and neutrophils is a characteristic feature [18–20]. In correlation with this cellular migration, there is an increase in the level of IL-8 in the serum, tissue and BAL of asthmatics. Similar to that in asthma, the involvement of IL-8 has been well investigated in adult respiratory distress syndrome (ARDS). However, the role of interleukin in the development of pneumonia is controversial [21]. Although, an increase in the level of IL-8 is a good indication of the inflammatory process, this information does not contribute much to clinical diagnosis. To our knowledge, no data is available on the production of IL-8 in BAL fluid from the same patient (i.e. infected and non-infected lung). Therefore this study is designed to measure the site-specific increase in the level of IL-8 in the lung of patients with bacterial pneumonia as compared to that of the non-smoking control group. The level of IL-8 mRNA and protein present in the BAL obtained from subsegmental bronchi of experimental and control group of patients were determined by RT-PCR assay and enzyme immunoassay respectively.

In this study we also determined the level of myeloperoxidase activity in the cells collected from 1 ml of BAL each from the infected and non-infected lung. Myeloperoxidase, a secreted heme protein, is an attractive candidate for monitoring phagocyte mediated cellular damage [22, 23].

Materials and methods

Patients

The study was performed on 36 patients with bacterial pneumonia who were admitted to the Chest Diseases Hospital in Kuwait. All patients underwent medical and laboratory examinations. The control group consisted of 17 non-smoking patients among which 9 patients were with chronic cough, 3 with hemoptysis and normal chest X-ray and 5 with old fibrotic shadows (Table 1).

Table 1. Characteristics of study population

	Bacterial pneumonia	Control
Number	36	17
Male/female	21/15	10/7
Age (yr; median and range)	34 (19–50)	29 (25–67)

Bronchoalveolar lavage (BAL)

BALs were obtained first from the area of known pneumonic consolidations on chest X-ray (infected lung) followed by BALs from other areas with no obvious infiltrate (non-infected lung) of the same patient. BAL fluids were collected from the pneumonic patient and the control group after admission to the hospital, using sterile techniques and routine respiratory care. The bronchoscope was advanced into a subsegmental bronchus. Lavage was performed using 20 ml aliquots of warmed normal saline, introduced by a syringe through bronchoscopic aspiration port. A total volume of 100–120 ml saline was infused sequentially and the volume of the lavage fluid retrieval (approximately 60 ml) was pooled and transferred immediately into sterile pre-chilled polypropylene tubes. The pooled fluid was then filtered through one layer of sterile gauze and centrifuged at 1500 rpm for 15 min at 4°C. Following centrifugation, 5 ml of supernatant was taken into a sterile polypropylene tube and stored at –70°C until assayed.

Isolation of cellular RNA

Total RNA was extracted from cells contained in 1 ml of the BAL obtained from the infected and non-infected lung, using the method of Chomczynski and Sacchi 1987 [24]. Briefly, the method is as follows. Cells were lysed in 0.5 ml of 4 M-guanidinium isothiocyanate. The lysates were then acidified by adding 80 µl of 3 M sodium acetate at pH 4.0. Subsequently, 0.5 ml of water saturated phenol and 0.1 ml chloroform were added to the cellular lysate followed by shaking at 4°C for 20 min. Lysates were spun in the cold for 15 min and the supernatants were collected and extracted again with phenol-chloroform. The supernatants were finally extracted with chloroform and the aqueous layer was collected. RNA was precipitated with absolute ethanol [25]. The precipitate was further centrifuged and the pellet was air-dried. The RNA pellet was then dissolved in 100 µl diethylpyrocarbonate (DEPC) treated water. Concentration of RNA was determined at 260/280 nm optical absorbance.

RT-PCR assay for IL-8 mRNA

Aliquots (2 µg) of total RNA were annealed with 250 ng of oligo dT primer by heating at 75°C for 10 min followed by its slow cooling to 37°C. Reverse transcription was carried out using 5 units of Avian Myeloma Virus (AMV) reverse

transcriptase and 20 units of RNA guard following the conditions described [26]. Reverse transcription reaction was carried out in 50 µl total volume and an aliquot of 5–10 µl from this cDNA was amplified for 35 cycles using the following PCR amplification parameters: denaturation 94°C × 30 sec, annealing 50°C × 30 sec and extension 74°C × 60 sec. The $MgCl_2$ was used at a concentration of 1.5 mmol/L.

The PCR amplification reaction was carried out in presence of 50 pmol each of upstream (5′-GGA ACC ATT CTC ACT GTG TG-3′) and down stream (5′-CTC TTC AAA AAC TTC TCC ACA A-3′) IL-8 specific primers using 1 unit of Amplitaq enzyme in a thermocycler. These primers were synthesized based on human IL-8 cDNA sequence information [27]. PCR products were analyzed on 10% polyacrylamide gel electrophoretically [28], stained with ethidium bromide and photographed with a gel documentation system (Stratagene). All experiments were carried out under RNase free conditions and the solutions and glassware were made RNase free with DEPC treatment and or by autoclaving. Heat sensitive solutions were made in DEPC treated and autoclaved water followed by their filtration through 0.45 µ size Millipore filters.

Enzyme immuno assay for IL-8

The concentrations of IL-8 in plasma and BAL fluid supernatants were assayed in duplicate, using a quantitative immunometric, 'sandwich' enzyme immunoassay technique with a detection limit of 4.7 pg/ml (Amersham, UK).

Measurement of BAL myeloperoxidase activity

Level of myeloperoxidase activity was estimated in the cells collected from 1 ml of BAL from the infected and non-infected lung. The method was essentially the same as described earlier [29]. Cells were pelleted by centrifugation at 4°C and were homogenized in 1 ml of hexadecyltrimethylammonium bromide buffer, containing 14 mM hexadecyltrimethylammonium bromide and 50 mM KPO4, pH 6.0. Samples were homogenized with polytron for 1 min and were kept cold on ice. The lysates were subsequently frozen in liquid nitrogen and thawed once. The lysates were then centrifuged for 2 min in cold at 14,000 rpm and the supernatants were used to estimate the level of MPO activity.

Aliquots of 20 µl supernatant were mixed with 980 µl of odianisidine HCl (sigma) solution containing 16.5 mg of odianisidine HCl, 90 ml of distilled water, 10 ml of KPO4 buffer, pH 6.0, and 50 µl of 1% H_2O_2. Absorbance was recorded at 415 nm, every 15 sec for 1 min using Beckman DU700 spectrophotometer. The enzyme activity was calculated (units/min/ml) by dividing the rate of the change in the absorbance by the extinction coefficient, 1.13×10^{-2}. Enzyme unit is defined as the conversion of 1 µmol of H_2O_2 per min per ml of alveolar lavage at room temperature. Under these conditions, the residual activity in the pellet was < 10%.

Statistical analysis

Commutations were performed using the Statview 4.02 statistical package with Macintosh Centris 650 computer. Results are expressed as means ± S.E.M. The differences between groups were analyzed by Student's *t*-test. The differences between the groups were considered significant if $p < 0.05$.

Results

In equal amounts of total cellular RNA, there was a significantly higher level of IL-8 mRNA in the infected lung as compared to that of the non-infected lung ($p < 0.001$; Fig. 1, lane 2). Before estimating the changes in the level of IL-8 mRNA, we characterized the identity of IL-8 PCR fragment (272 bp). For this purpose we employed HindIII restriction enzyme. This enzyme cut the IL-8 PCR fragment (272 bp)

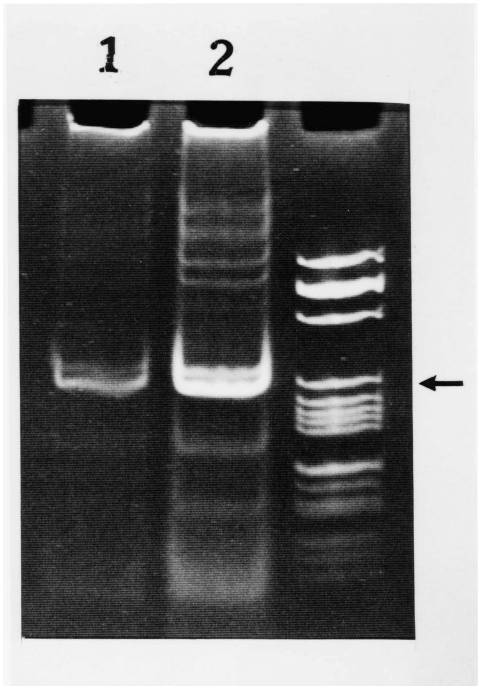

Fig. 1. Ethidium bromide stained gel picture showing the level of IL-8 mRNA in the left lung (lane 1) and the right lung (lane 2). Each lane exhibits the level of IL-8 mRNA in an equivalent of 200 ng total cellular RNA after PCR amplification for 35 cycles. Right most flanking lane (unlabeled) shows the PBR322xHaelll size marker and arrow indicates the position of 272 hp IL-8 PCR fragment.

into 193 and 81 bp fragments of expected size (data not shown), proving the identity of IL-8 PCR fragment (272 bp).

The concentrations of IL-8 in BAL fluids taken from patients with bacterial pneumonia were always high as compared to control group ($p < 0.0001$; Fig. 2). Furthermore, in all patients the levels of IL-8 in BAL obtained from the infected lung were 6 fold higher than those from the non-infected lung 130.6 ± 6 to 194.32 ± 54 pg/ml, n = 36 ($p < 0.0001$). This pattern of increased expression of IL-8 mRNA and protein was consistent with MPO activity in the lavages. In the cells from the equal amount (1 ml) of alveolar lavages there was 4.5 times more MPO activity (9.0 units/min/ml) in the right lung as compared to the level (2.0 units/min/ml) in the left lung (Fig. 3).

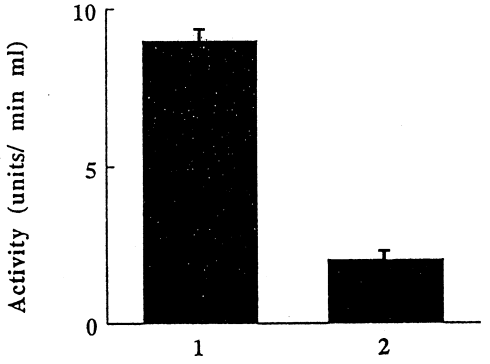

Fig. 3. Bar diagram shows the level of MPO activity in the left lung (bar 1) and in the right lung (bar 2). The activity level is obtained from the equal volume (1 ml) of alveolar lavages from both lungs. Enzyme unit has been defined in the Materials and methods section and is expressed as units/min ml of ravage shown on y-axis.

Discussion and conclusion

Recent reports have considered IL-8 as the most potent and major PMN chemoattractant factor in lung diseases [13–17], including ARDS and pneumonia [21], cystic fibrosis [30], human immunodeficiency virus (HIV)-infected patients with *Pneumocystis carinii* pneumonia, bacterial pneumonia, or tuberculosis [31]. Several studies have convincingly shown that IL-8 plays a key role in the pathobiology of asthma [18–20]. Presence of IL-8 has been demonstrated in the bronchioalveolar fluid (BAL) of the patients with asthma [13–15]. Furthermore, in asthmatics, there was an increase in the level of free and complex IL-8 in the blood as well as the bronchial mucosa [32], suggesting that free IL-8 may have a role in the activation of eosinophils. Various other convincing studies suggest that IL-8 is an eosinophil and neutrophil chemoattractant [33–36]. It has been shown that IL-8 plays a major role in adult respiratory syndrome (ARDS) [37–39]. On the other hand, certain studies could not find a correlation between the percentage of PMNs and the concentration of IL-8 in BAL fluid of patients with ARDS [21], suggesting that in addition to IL-8 there may be other chemoattractant agents that are involved in transendothelial migration of PMNs. Several investigators have demonstrated that BAL fluids obtained from patients with pulmonary infection, contain potent chemotactic factors, such as the complement peptide C5a and leukotriene-B4 (LTB4) [40].

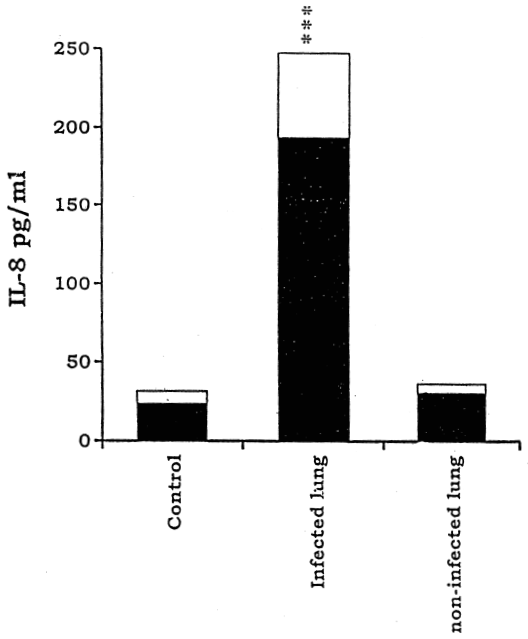

Fig. 2. IL-8 concentration (pg/ml) in BAL fluid of patients with bacterial pneumonia (comparison between infected and non-infected lung). Values represent the mean ± S.E. $p < 0.0001$.

In guinea pigs, exogenous IL-8 administration has been shown to recruit neutrophils in the airway lumen [41]. In addition, *in vivo* and *in vitro* studies have shown that IL-8 induces the release of T-lymphocyte chemoattractants from neutrophil [35]. *In vivo* studies in mice with a targeted deletion of IL-8 receptor homologue has shown that the total number of recruited cells to the airway lumen following a single antigen challenge was significantly low as compared to the wild type [42].

In consistent with the above studies, in the present study, high concentrations of IL-8 were found in BAL fluids taken from the infected lung of patients with bacterial pneumonia. These results are in agreement with other investigators [21, 43], who reported high levels of IL-8 in BAL fluids of patients with different lung diseases. Since alveolar macrophages are the major source of IL-8 in the lung, the local production of IL-8 by these cells may be responsible for the recruitment of PMNs into the pulmonary interstitial or air space in a variety of lung diseases. Further studies are required to determine the relationship between the severity of

lung diseases and the alteration of IL-8 in BAL fluids. Nevertheless, the data presented clearly show that the concentration of IL-8 in BAL fluid from pneumonic patients increased in the infected lung.

In the lungs, IL-8 appears to be the primary chemoattractant for neutrophils [43]. In addition to various cell types [44], IL-8 is also synthesized and released by neutrophils [45]. Thus, neutrophils contribute to the recruitment of additional neutrophils in an autocrine manner, by the synthesis and release of IL-8. Several studies suggest that IL-8 also functions as a chemoattractant for eosinophils [33–36]. It has been shown that major basic protein (MBP), a 13.9 kD protein located in the crystalloid core of eosinophil secondary granules, stimulates the production of IL-8 through transcriptional and post-transcriptional events [46, 47]. In neutrophils, MBP stimulated IL-8 production occur post-transcriptionally through stabilization of IL-8 mRNA [47].

In a recent study it has been noticed that type specific consequences of lung infection may be due to the type specific differences in the induction of cytokines by various infectious agents [48]. In contrast to type 5 adenovirus, type 7 adenovirus stimulated the production of IL-8 in human lung alveolar epithelial cell line (A549 cells) and primary human fetal lung fibroblasts (GM5387 cells). The regulation of IL-8 production, in these cells, occurred at the transcriptional level and at the level of message stability [48]. While adenovirus type 7 increased endogenous IL-8 specific mRNA, both serotypes (type 7 and type 5) enhanced stabilization of IL-8 mRNA [48].

The data presented in our study shows that there was a significant increase in the level of IL-8 mRNA in the infected lung as compared to its level in the non-infected lung ($p < 0.001$). In correlation with the increase in mRNA, IL-8 protein concentrations in BAL fluids from the infected lung were 6 fold higher than those taken from the non-infected lung ($p < 0.0001$). The mechanism of IL-8 specific mRNA increase (transcriptional or post-transcriptional) in this study need to be elucidated.

The techniques that are commonly used to quantitate inflammatory cells during the development of various diseases in the lungs include histological analysis and *ex vivo* radiolabeling of leucocytes and quantification of their accumulation in the lungs by counting. These techniques, however, are labor and time intensive and have practical limitations [49–53]. To overcome the limitations of histological and radiolabeling studies, in the present study, we have measured the myeline peroxidase (MPO) activity, in order to quantify the neutrophil accumulation.

The pattern of MPO activity in the BALs (4.5 fold more MPO activity in the infected lung as compared to that of the non-infected lung) was consistent with the level of IL-8 mRNA and protein. The results of the present study, therefore, indicate a site-specific involvement of IL-8 in the pathogenesis of pneumonia.

References

1. Hiemstra PS: Role of neutrophils and mononuclear phagocytes in host defense and inflammation. JIFCC 5: 224–231, 1993
2. Goldstein IM, Hoffstein S, Gallin JI, Weissmann G: Mechanisms of lysosomal enzyme release from human leukocytes: Microtubule assembly and membrane fusion induced by a component of complement. Proc Natl Acad Sci USA 70: 2916–2920, 1973
3. Becker EL, Showell HJ, Henson PM, Hsu LS: The ability of chemotactic factors to induce lysosomal enzyme release. I. The characteristics of the enzyme release, importance of surfaces and the relation of enzyme release to chemotactic responsiveness. J Immunol 12: 2047–2054, 1974
4. Zurier RB, Hoffstein S, Weissmann G: Cytochalasin B: Effect of lysosomal enzyme release from human leukocytes. Proc Natl Acad Sci USA 70: 844–848, 1973
5. Showell HJ, Freer RJ, Zigmond SH, Schiffmann E, Aswankumar S, Corcoran B, Becker EL: The structure activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. J Exp Med 143: 1154–1169, 1976
6. Becker EL: Some interrelations of neutrophil chemotaxis, lysosomal enzyme secretion, and phagocytosis as revealed by synthetic peptides. Am J Pathol 85: 385–394, 1976
7. Bainton DF, Ullyot JL, Farquhar MG: The development of neutrophilic polymorphonuclear leukocytes in human bone marrow: Origin and content of azurophil and specific granules. J Exp Med 134: 907–1000, 1971
8. Bainton DF: Sequential degranulation of the two types of polymorphonuclear leukocyte granules during phagocytosis of microorganisms. J Cell Biol 58: 249–261, 1973
9. Kudo C, Yamashita T, Araki A, Terashita M, Watanabe T, Atsumi M, Tamura M, Sendo F: Modulation of *in vivo* immune response by selective depletion of neutrophils using a monoclonal antibody, RP-3. I. Inhibition by RP-3 treatment of the priming and effector phases of delayed type hypersensitivity to sheep red blood cells in rats. J Immunol 150: 3728–3738, 1993
10. Lewis RA, Austin KF: The biologically active leukotrienes. J Clin Invest 73: 889–897, 1984
11. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K: Properties of the novel proinflammatory supergene 'intercrine' cytokine family. Annu Rev Immunol 9: 617–648, 1991
12. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K: The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science 243: 1464–1466, 1989
13. Virchow JC, Kroegel C, Walker C, Matthys H: Inflammatory determinants of asthma severity: Mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. J Allergy Clin Immunol 98: S27–S33, 1996
14. Chanez P, Enander I, Jones I, Godard P, Bousquet J: Interleukin 8 in bronchoalveolar lavage of asthmatic and chronic bronchitis patients. Int Arch Allergy Immunol 111: 83–88, 1996
15. Folkard SG, Westwick J, Millar AB: Production of interleukin-8, RANTES and MCP-1 in intrinsic and extrinsic asthmatics. Eur Respir J 10: 2097–2104, 1997
16. Shute JK: IL-8 is a potent eosinophil chemoattractant. Clin Exp Allergy 24: 203–206, 1994
17. Nocker RE *et al.*: Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. Int Arch Allergy Immunol 109: 183–191, 1996
18. Robinson DS *et al.*: Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 326: 298–304, 1992
19. Sur S, Kita H, Gleich GJ, Chenier TC, Hunt LW: Eosinophil recruitment is associated with IL-5, but not with RANTES, twenty-four hours after allergen challenge. J Allergy Clin Immunol 97: 1272–1278, 1996

20. Bousquet JP *et al*.: Eosinophilic inflammation in asthma. N Engl J Med 323:1033–1039, 1990
21. Chollet-Martins S, Montravers P, Gibert C, Elbim MC, Desmonts JM, Fagon JY, Gougerot - Pocidalo MA: High levels of interleukin-8 in the blood and alveolar spaces of patients with pneumonia and adult respiratory distress syndrome. Infect Immun 61: 4553–4559, 1993
22. Klebanoff SJ: Oxygen metabolism and toxic properties of phagocytes. Ann Intern Med 93: 480–489, 1980
23. Hurst JK, Barrette WC Jr: Leukocytic oxygen activation and microbicidal oxidative toxins. CRC Crit Rev Biochem Mol Biol 24: 271–328, 1989
24. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. Anal Biochem 162: 156–159, 1987
25. Maniatis T, Fritsch EF, Sambrook J: In: Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982
26. Khan I, Collins SM: Altered expression of sodium pump isoforms in the inflamed intestine of Trichinella spiralis-infected rats. Am J Physiol 264: G1160–G1168, 1993
27. Mukaida N, Shiroo M, Matsushima K: Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 143: 1366–1371, 1989
28. Khan I, Tabb T, Grafield RE, Grover AK: Polymerase chain reaction assay of mRNA using 28s rRNA as internal standard. Neurosci Lett 147: 114–117, 1992
29. Bradley PP, Priebat DA, Christensen RD, Rothstein G: Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78: 206–209, 1982
30. Dean TP, Dai Y, Shute JK, Church MK, Warner JO: Interleukin-8 concentrations are eluted in bronchoalveolar lavage, sputum and sera of children with cystic fibrosis. Pediatr Res 34: 159–161, 1993
31. Grunewald T, Schuler-Maue W, Ruf B: Interleukin-8 and granulocyte colony-stimulating factor in bronchoalveolar lavage fluid and plasma of human immunodeficiency virus infected patients with pneumocystis carinii pneumonia, bacterial pneumonia, or tuberculosis. J Infect Dis 168: 1077–1078, 1993
32. Shute JK, Vrugt B, Lindley IJD, Holgate ST, Bron A, Aalbers R, Djukanovic R: Free and complexed interleukin-8 in blood and bronchial mucosa in asthma. Am J Respir Crit Care Med 155: 1877–1883, 1997
33. Schratzberger P, Dunzendorfer S, Reinisch N, Kähler CM, Wiedermann, CJ: Interleukin-8–induced human peripheral blood B-lymphocyte chemotaxis *in vitro*. Immunol Lett 58: 167–170, 1997
34. Shute JK: IL-8 is a potent eosinophil chemoattractant. Clin Exp Allergy 24: 203–206, 1994
35. Taub DD, Anver M, Oppenheim JJ, Longo DL, Murphy WJ: T -lymphocyte recruitment by interleukin-8. IL-8-induced degranulation of neutrophils releases potent chemoattractants for human T lymphocytes both *in vitro* and *in vivo*. J Clin Invest 97: 1931–1941, 1996
36. Wang JM, Xu L, Murphy WJ, Taub DD, Chertov O: IL-8–induced T-lymphocyte migration: Direct as well as indirect mechanisms. Methods 10: 135–144, 1996
37. Miller EJ, Cohen AB, Nagao S, Griffith D, Maunder RJ, Martin TR, Weiner-Kronish JP, Sticherling M, Christophers E, Mattay MA: Elevated levels of NAP-1/interleukin-8 are present in the airspace of patients with adult respiratory distress syndrome and are associated with increased mortality. Am Rev Respir Dis 146: 427–432, 1992
38. Matsumoto T, Yokoi K, Mukaida N, Harada A, Yamashita J, Watanabe Y, Matsushima K: Pivotal role of interleukin-8 in the acute respiratory distress syndrome and cerebral reperfusion injury. J Leukocyte Biol 62: 581–587, 1997
39. Mukaida N, Matsumoto T, Yokoi K, Harada A, Matsushima K: Inhibition of neutrophil-mediated acute lung inflammation injury by an antibody against interleukin-8 (IL-8). Inflamm Res 47: S151–S157, 1998
40. Hopkins H, Stull T, Von Essen SG, Robbins RA, Rennard SI: Neutrophil chemotactic factors in bacterial pneumonia. Chest 95:1021–1027, 1989
41. Fujimura M *et al*.: Sensory neuropeptides are not directly involved in bronchial hyperresponsiveness induced by interleukin-8 in guinea-pigs *in vivo*. Clin Exp Allergy 26: 357–362, 1996
42. De Sanctis GT, MacLean JA, Qin S, Wolyniec WW, Grasemann H, Yandava CN, Jiao A, Noonan T, Stein-Streilein J, Green FHY, Drazen JM: Interleukin-8 receptor modulates IgE production and B-cell expansion and trafficking in allergen-induced pulmonary inflammation J Clin Invest 4: 507–515, 1999
43. Kunkel SL, Standiford T, Kasahara K, Strieter M: Interleukin-8 (IL-8): The major neutrophil chemotactic factor in the lung. Exp Lung Res 17: 17–23, 1991
44. Taub DD, Oppenheim JJ: Chemokines, inflammation and the immune system. Ther Immunol 1: 229–246, 1994
45. Strieter RM, Kasahara K, Allen RM, Standiford TJ, Rolfe MW, Becker FS, Chensue SW, Kunkel SL: Cytokine-induced neutrophil-derived interleukin-8. Am J Pathol 141: 397–407, 1992
46. Kita H, Adolphson CR, Gleich GJ: Biology of eosinophils. In: E. Middleton Jr, E.F. Ellis, J.W. Yuninger, C.A. Reed, N.F. Adkinson Jr, W.W. Busse (eds). Allergy- Principles and Practice, vol. 1. Mosby, St. Louis, 1998, pp 242–260.
47. Stoeckle MY: Post-transcriptional regulation of gro, and IL-8 mRNAs by IL-1. Nucleic Acids Res 19: 917–920, 1991
48. Booth JL, Metcalf JP: Type-specific induction of interleukin-8 by adenovirus. Am J Respir Cell Mol Biol 21: 521–527, 1999
49. Sanz MJ, Weg VB, Bolanowski MA, Nourshargh S: IL-1 is a potent inducer of eosinophil accumulation in rat skin: Inhibition of response by a platelet-activating factor antagonist and an anti-human IL-8 antibody. J Immunol 154: 1364–1373, 1995
50. Faccioli LH, Nourshargh S, Moqbel R, Williams FM, Sehmi R, Kay AB, Williams TJ: The accumulation of ^{111}In-eosinophils induced by inflammatory mediators, *in vivo*. Immunology 73: 222–227, 1991
51. Walker C, Rihs S, Braun RK, Betz S, Bruijnzeel PL: Increased expression of CD11b and functional changes in eosinophils after migration across endothelial cell monolayers. J Immunol 150: 4061–4071, 1993
52. Berends C, Dijkhuizen B, de Monchy JGR, Gerritsen J, Kauffman HF: Induction of low density and up-regulation of CD11b expression of neutrophils and eosinophils by dextran sedimentation and centrifugation. J Immunol Meth 167: 183–193, 1994
53. Youssef PP, Mantzioris BX, Roberts-Thomson PJ, Ahern MJ, Smith MD: Effects of *ex vivo* manipulation on the expression of cell adhesion molecules on neutrophils. J Immunol Meth 186: 217–224, 1995