Molecular and Cellular Biochemistry **234/235:** 225–228, 2002.

Endogenous regulation of the acute inflammatory response

Peter A. Ward and Alex B. Lentsch
Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Abstract

The acute inflammatory response has been triggered in rat lungs by deposition of IgG immune complexes. The inflammatory reaction triggered is highly tissue damaging and requires activation of NF-κB with ensuing generation of chemokines and cytokines. Endogenous generation of IL-10 and IL-13 as well as secretory leukocyte protease inhibitor (SLPI), significantly regulates this inflammatory response. IL-10 and IL-13 attenuate NF-κB activation by interfering with breakdown of IκBα, while SLPI likewise suppresses NF-κB activation, but by interfering with breakdown of IκBβ. Antibody induced blockade of IL-10, IL-13 or SLPI enhances NF-κB activation in lung and exacerbates the lung inflammatory response and injury. These data indicate that endogenous IL-10, IL-13 and SLPI are important regulators of the inflammatory response by reducing gene activation with resultant generation of peptide mediators/cytokines and chemokines. (Mol Cell Biochem **234/235**: 225–228, 2002)

Key words: NF-κB, inflammation, lung injury, neutrophils

Introduction

The inflammatory response in lungs of rodents has been extensively studied and the pathways leading to critical production of inflammatory mediators elucidated. The intrapulmonary deposition of IgG immune complexes in rats induces an intense inflammatory response that is characterized by alveolar edema and hemorrhage and an intense accumulation of neutrophils [1]. Tissue injury is attributable to products of both neutrophils and lung macrophages and involving the generation of toxic oxygen products and release of proteases. These inflammatory reactions are known to be neutrophil and complement dependent and also require the participation of cytokines (IL-1, TNFα) and chemokines (the CXC chemokines, MIP-2 and CINC and the CC chemokines, MIP-1α and MIP-1β) [1–6]. The pathways by which products of stimulated alveolar macrophages and alveolar epithelial cells activate vascular endothelial cells are shown in Fig. 1. TNFα generated by each type of cell can activate nearby vascular endothelial cells, causing upregulation of adhesion molecules (ICAM-1 and E-selectin), while other products (MIP-2) can cause chemoattraction of blood neutrophils into the interstitial and alveolar spaces. These inflammatory reactions are tightly regulated. In fact, the influx of neutrophils and the vascular leak peak at 4 h and then promptly diminish. This review will discuss some of the endogenous mediators that regulate these acute inflammatory responses.

Regulation of activation of NF-κB and IL-10 and IL-13

The dimeric (chiefly heterodimeric) complex of NF-κB is well-known to be important in bringing about gene activation and generation of the cytokines and chemokines mentioned above [7, 8]. NF-κB is located in the cytoplasm of a variety of cell types and is linked to the inhibitor, IκB, which prevents the translocation of NF-κB to the nucleus where it has access to DNA promoter sites. When IκB undergoes hydrolysis by the 26S proteasome, NF-κB is then free to translocate to the nucleus and initiate gene activation.

In the IgG immune complex model of acute lung injury, two waves of NF-κB activation occur, one at 0.5 h involving lung macrophages, and a second wave peaking at 4 h and involving other lung cell types [9, 10]. While it is well established that *in vitro* incubation of macrophages with TNFα or airway instillation of TNFα involves activation (nuclear

Address for offprints: P. Ward, University of Michigan Health Systems, Department of Pathology, 1301 Catherine Road, Medical Science I Building, Room M5240, Ann Arbor, MI 48109, USA (E-mail: pward@umich.edu)

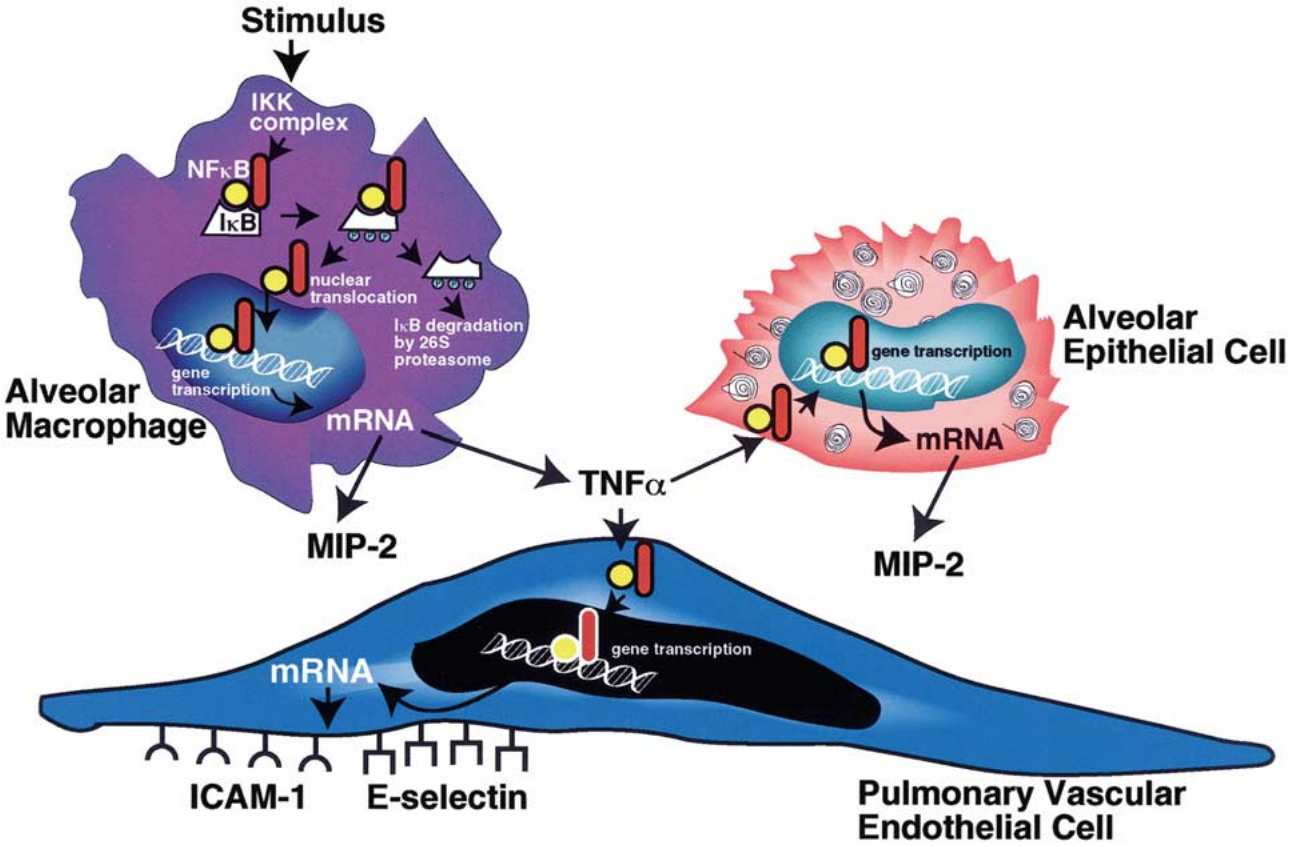

Fig. 1. Possible pathways by which products of activated alveolar macrophages and alveolar epithelial cells activate endothelial cells and cause neutrophil accumulation.

translocation) of NF-κB, we have found that IL-10 and IL-13, which are produced during the induction of lung injury as described above, induce powerful inhibition of NF-κB activation [9, 10]. The mechanism of this inhibition has been linked to the preservation of IκBα, which fails to undergo hydrolysis in the presence of either IL-10 or IL-13. Conversely, if either IL-10 or IL-13 is blocked *in vivo* by the airway instillation of antibody, NF-κB activation is intensified, and lung levels of mediators such as TNFα and MIP-2 are elevated above those found in otherwise unmanipulated lungs undergoing IgG immune complex damage [11, 12]. The precise mechanisms by which IL-10 and IL-13 are able to suppress hydrolysis of IκBα are under intense investigation. Both IL-10 and IL-13 have been shown to prevent the DNA-binding of NF-κB [13, 14]. In addition, IL-10 is thought to prevent IκBα degradation by suppressing activation of IκB kinase (IKK) activity [13]. Utilizing these mechanisms, endogenous production of IL-10 and IL-13 powerfully regulates the inflammatory process, preventing excessive tissue damage and providing a balance that allows lung repair after this intense inflammatory response.

Role of secreted leukocyte protease inhibitor (SLPI) in lung inflammatory responses

SLPI was originally identified as a serine protease inhibitor secreted by cervical epithelium, but it is now evident that this inhibitor can be produced in a variety of tissues and by a variety of cell types [15–20]. SLPI is a 12 kDa single chain protein containing two domains, one of which contains Leu^{72} which is a critical binding site for susceptible serine proteases (chymotrypsin, elastase and trypsin) [21, 22].

When exogenous human recombinant SLPI is instilled into the airways of rats undergoing immune complex-induced injury, there is inhibition of the inflammatory response as defined by increases in the lung permeability index, in numbers of neutrophils recruited into lung, and by levels in bronchoalveolar (BAL) fluids of TNFα, MIP-2 and CINC [23]. This inhibition is dose-dependent on the amount of SLPI used. Most importantly, the presence of exogenous SLPI markedly reduces the level of NF-κB activation, whereas another endogenous protease inhibitor, tissue inhibitor of metalloproteases-2, has no protective effects [24]. In addi-

tion, these studies demonstrated that the exogenous addition of SLPI in amounts that are protective in the animal model employed fail to demonstrate any inhibition of p42/p44 isoforms of mitogen activate protein kinases (MAPK). In the inflammatory lung model employed, SLPI is endogenously produced and can be detected by Western blot analysis in BAL fluids [25]. Under these experimental conditions, immunostaining has revealed the induction of SLPI in alveolar epithelial cells, in lung macrophages and in vascular endothelial cells. Thus, in the activated lung, there appear to be many sources of SLPI. If antibody to SLPI is instilled into the airways of rats undergoing inflammatory injury, NF-κB activation is intensified, in concert with enhanced injury of lung [24, 25]. Thus, endogenous SLPI also appears to be an important natural regulator of the inflammatory response.

The mechanism by which SLPI impairs NF-κB activation in the lung is distinctly different from the manner by which IL-10 and IL-13 inhibit NF-κB activation. SLPI does not affect the breakdown of IκBα in these lung inflammatory reactions; rather, SLPI prevents by an unknown mechanism the breakdown of IκBβ, another important regulator of NF-κB activation [24]. As might be expected, TIMP-2 fails to show similar functionality.

The availability of mutant forms of human SLPI has provided additional structure-function information related to SLPI. The substitution of Leu^{72} of SLPI with Gly or Phe attenuates the protective effects of SLPI in the lung injury model (as defined by the vascular permeability index or by BAL neutrophil numbers), while Lys^{72} SLPI is at least as protective as Leu^{72} SLPI (the wild-type form of SLPI) [26]. In parallel, Phe^{72} SLPI suppresses NF-κB activation in lung, similar to wild-type SLPI. Precisely what serine protease in the inflamed lung is being inhibited by SLPI is unclear, and it is also uncertain if SLPI is interacting with an extracellular or intracellular serine protease. Another serine protease inhibitor (PI), α_1PI, which is known to be abundantly present in both serum and in lung BAL fluids, does not when instilled into lungs similar to the use of SLPI lead to inhibition of NF-κB activation or protection from immune complex-induced acute lung injury. Why under these circumstances α_1PI is not protective is unknown. It is possible that α_1PI, which is considerably larger (53 kDa), may not have access to the intracellular compartment of cells, if this is indeed the manner by which SLPI is functional. These data indicate that SLPI is another important endogenous regulator of NF-κB activation during the acute inflammatory response.

In summary, regulation of the acute inflammatory response in lung occurs in part by increased endogenous production of IL-10, IL-13 and SLPI. Each of these mediators function as inhibitors of NF-κB activation. Thus, NF-κB appears to be critical for the induction of the acute inflammatory response in the lung and the expression of IL-10, IL-13 and SLPI is necessary for intrinsic control of this response.

References

1. Johnson KJ, Ward PA: Acute immunologic pulmonary alveolitis. J Clin Invest 54: 349–357, 1974
2. Warren JS, Yabroff KR, Remick DG, Kunkel SL, Chensue SW, Kunkel RG, Johnson KJ, Ward PA: Tumor necrosis factor participates in the pathogenesis of acute immune complex alveolitis in the rat. J Clin Invest 84: 1873–1882, 1989
3. Warren JS: Intrapulmonary interleukin 1 mediates acute immune complex alveolitis in the rat. Biochem Biophys Res Commun 175: 604–610, 1991
4. Shanley TP, Schmal H, Warner RL, Schmid E, Friedl HP, Ward PA: Requirement for C-X-C chemokines (macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant) in IgG immune complex-induced lung injury. J Immunol 158: 3439–3448, 1997
5. Shanley TP, Schmal H, Friedl HP, Jones ML, Ward PA: Role of macrophage inflammatory protein-1α (MIP-1α) in acute lung injury in rats. J Immunol 154: 4793–4802, 1995
6. Bless NM, Huber-Lang M, Guo RF, Warner RL, Schmal H, Czermak BJ, Shanley TP, Crouch LD, Lentsch AB, Sarma V, Mulligan MS, Freidl HP, Ward PA: Role of CC chemokines (MIP-1β, MCP-1, RANTES) in acute lung injury in rats. J Immunol 164: 2650–2659, 2000
7. Ghosh S, May MJ, Kopp EB: NF-κB and Rel proteins: Evolutionarily conserved mediators of immune responses. Annu Rev Immunol 16: 225–260, 1998
8. Pahl HL: Activators and target genes of Rel/NF-κB transcription factors. Oncogene 18: 6853–6866, 1999
9. Lentsch AB, Shanley TP, Sarma V, Ward PA. *In vivo* suppression of NF-κB and preservation of IκBα by interleukin-10 and interleukin-13. J Clin Invest 100: 2443–2448, 1997
10. Lentsch AB, Czermak BJ, Bless NM, Ward PA. NF-κB activation during IgG immune complex-induced lung injury. Requirements for TNF-α and IL-1β but not complement. Am J Pathol 152: 1327–1336, 1998
11. Shanley TP, Schmal H, Friedl HP, Jones ML, Ward PA: Regulatory effects of intrinsic IL-10 in IgG immune complex- induced lung injury. J Immunol 154: 3454–3460, 1995
12. Lentsch AB, Czermak BJ, Jordan JA, Ward PA: Regulation of acute lung inflammatory injury by endogenous IL-13. J Immunol 162: 1071–1076, 1999
13. Schottelius AJ, Mayo MW, Sartor RB, Baldwin AS Jr: Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. J Biol Chem 274: 31868–31874, 1999
14. Manna SK, Aggarwal BB: IL-13 suppresses TNF-induced activation of nuclear factor-kappa B, activation protein-1, and apoptosis. J Immunol 161: 2863–2872, 1998
15. Schill WB, Wallner O, Schiessler H, Fritz H: Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus. Experientia 34: 509–510, 1978
16. Lee CH, Igarashi Y, Hohman RJ, Kaulbach H, White MV, Kaliner MA: Distribution of secretory leukoprotease inhibitor in the human nasal airway. Am Rev Respir Dis 147: 710–716, 1993
17. Fryksmark U, Jannert M, Ohlsson K, Tegner H, Wihl JA: Secretory leukocyte protease inhibitor in normal, allergic and virus induced nasal secretions. Rhinology 27: 97–103, 1989
18. Fryksmark U, Ohlsson K, Polling A, Tegner H: Distribution of antileukoprotease in upper respiratory mucosa. Ann Otol Rhinol Laryngol 91: 268–271, 1982

19. Moriyama A, Shimoya K, Kawamoto A, Hashimoto K, Ogata I, Kunishige I, Ohashi K, Azuma C, Saji F, Murata Y: Secretory leukocyte protease inhibitor (SLP) concentrations in seminal plasma: SLPI restores sperm motility reduced by elastase. Mol Hum Reprod 4: 946–950, 1998
20. Ohlsson K, Bjartell A, Lilja H: Secretory leucocyte protease inhibitor in the male genital tract: PSA-induced proteolytic processing in human semen and tissue localization. J Androl 16: 64–74, 1995
21. Thompson RC, Ohlsson K. Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. Proc Natl Acad Sci USA 83: 6692–6696, 1986
22. Eisenberg SP, Hale KK, Heimdal P, Thompson RC. Location of the protease-inhibitory region of secretory leukocyte protease inhibitor. J Biol Chem 265: 7976–7981, 1990
23. Mulligan MS, Desrochers PE, Chinnaiyan AM, Gibbs DF, Varani J, Johnson KJ, Weiss SJ. *In vivo* suppression of immune complex-induced alveolitis by secretory leukoproteinase inhibitor and tissue inhibitor of metalloproteinases 2. Proc Natl Acad Sci USA 90: 11523–11527, 1993
24. Lentsch AB, Jordan JA, Czermak BJ, Diehl KM, Younkin EM, Sarma V, Ward PA. Inhibition of NF-κB activation and augmentation of IκBα by secretory leukocyte protease inhibitor during lung inflammation. Am J Pathol 154: 239–247, 1999
25. Gipson TS, Bless NM, Shanley TP, Crouch LD, Bleavins MR, Younkin EM, Sarma V, Gibbs DF, Tefera W, McConnell PC, Mueller WT, Johnson KJ, Ward PA: Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. J Immunol 162: 3653–3662, 1999
26. Mulligan MS, Lentsch AB, Huber-Lang M, Guo RF, Sarma V, Wright CD, Ulich TR, Ward PA: Anti-inflammatory effects of mutant forms of secretory leukocyte protease inhibitor. Am J Pathol 156: 1033–1039, 2000