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Macrophage inflammatory protein-1 alpha expression in non-neoplastic and neoplastic lung tissue

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Abstract The chemokines are members of a bipartite superfamily of soluble proteins that have been implicated in a wide range of acute and chronic inflammatory processes, as well as other immunoregulatory functions. Macrophage inflammatory protein-1 alpha (MIP-1α) belongs to the C-C subfamily of these chemokines and is primarily a potent chemoattractant and activator of monocytes. MIP-1α is also thought to play a role in host defence. We examined the expression of MIP-1α in normal lung, inflammatory lung tissue and lung cancer cells by the immunoperoxidase method using a MIP-1α monoclonal antibody. MIP-1α protein was found to be expressed not only by alveolar macrophages, but also by bronchial ciliated cells, hyperplastic alveolar type II cells and activated fibroblasts surrounding malignant tissue. Of 33 cases of lung cancer, 23 (70%) expressed MIP-1α. These observations suggest that lung cancer cells, non-neoplastic alveolar type II cells and fibroblasts can participate in inflammatory cell recruitment via the production of MIP-1α. Tumour derived MIP-α may also affect the interaction between lung cancer and host inflammatory cells.

Key words Macrophage inflammatory protein-1 alpha · Monoclonal antibody · Alveolar type II cell · Fibroblast · Lung cancer

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Introduction

Macrophage inflammatory protein-1 (MIP-1) was originally described as an endotoxin-induced macrophage product with both neutrophil chemoattractant and activating properties [25]. Based on amino acid sequence similarities, MIP-1 has been placed into a group of pro-inflammatory cytokines, designated the chemokine family [14]. The family has been further divided into the C-C and C-X-C families depending upon whether the first two conserved cysteine amino acid residues are in juxtaposition (C-C) or separated by one amino acid (C-X-C). The C-X-C chemokines include interleukin-8 (IL-8), platelet factor 4, and β-thromboglobulin whereas the C-C chemokines include MIP-1, monocyte chemoattractant protein-1 (MCP-1) and RANTES (regulated upon activation, normal T cell expressed and secreted) [14]. MIP-1 was originally shown to activate polymorphonuclear leukocytes both in vivo and in vitro [24, 25], but recent studies using purified MIP-1 have suggested that the chemotactic target cells are actually T cells and macrophages rather than neutrophils [13]. MIP-1 consists of two peptides, MIP-1α and MIP-1β. MIP-1α and MIP-1β perform similar functions that include the induction of inflammatory infiltrates, but have disparate immunological effects. For example, MIP-1α inhibits haematopoietic stem cell growth, whereas MIP-1β counteracts the ability of MIP-1α to suppress stem cell growth. Of these two proteins, MIP-1α exerts greater pro-inflammatory effects than MIP-1β [21].

Recently, it has been shown that alveolar type II cells and cancer cell lines are capable of producing cytokines that include MCP-1 [9, 20] and IL-8 [10, 19]. MIP-1α has also been found in alveolar macrophages [1] and fibroblasts in interstitial lung disease [5], but has not been identified in neoplastic lung tissue. In the present study, we describe the distribution of MIP-1α protein in neoplastic and non-neoplastic lung tissue using a monoclonal antibody directed against MIP-1α that is effective in formalin-fixed, paraffin-embedded specimens.

Materials and methods

Surgically resected lung specimens from 33 patients with bronchogenic carcinoma treated at Shiga University of Medical Science in 1993 were studied. For each case, neoplastic tissue and corresponding non-neoplastic lung tissue in close proximity to the tumour were analyzed. Histologically normal lung tissue was also examined and compared with both neoplastic and inflamed lung tissue. No patient had received radiotherapy or chemotherapy prior to surgery.

Representative tissue specimens were fixed in 10% formalin, embedded in paraffin, routinely processed for histopathological examination, and stained with haematoxylin and eosin. Immunoperoxidase staining was also performed on paraffin-embedded tissue sections. Primary antibodies used in this study were anti-MIP-1α antibody (lambda 78 delta) developed by one of us, recognizing an antigen epitope stable to formalin fixation [2], anti-surfactant protein A antibody (SP-A) (PE 10, DAKO Japan, Kyoto, Japan) [11] and anti-alpha smooth muscle actin (1A4, Sigma, Mo., USA) [17]. Sections (5 μm) thick were deparaffinized and endogenous peroxidase activity was blocked using 0.3% hydrogen peroxidase in methanol. Sections were then incubated with the primary monoclonal antibodies at room temperature, followed by incubation with biotin-labelled secondary antibody. Finally, sections were subjected to peroxidase conjugated with streptavidin using a commercially available kit (Universal quick stain kit, DAKO). Peroxidase activity was detected using the chromogen diaminobenzidine (DAKO). Nuclei were counterstained with methyl green. Prior to MIP-1α immunostaining, sections were treated with 0.1% trypsin in 0.2% calcium chloride for 15 min at 37° following deparaffinization. Ganglion cells and smooth muscle cells within small intestinal walls were used as positive controls for MIP-1α and smooth muscle actin immunostains. For SP-A immunostaining, alveolar type II pneumocytes found in non-neoplastic lung tissue was used as an internal positive control. Negative controls were performed by substituting the primary antibody with phosphate buffered saline.

For non-neoplastic lung tissue, the staining intensity was rated as strongly positive (S), weakly positive (W) or negative (N). The staining of malignant cells was scored as follows: staining of the majority (>70%) of tumour cells (+++); staining of 30–70% of tumour cells (++); staining in fewer than 30% of tumour cells (+).

Results

These results of immunohistochemical studies of non-neoplastic tissue are summarized in Table 1. Expression of MIP-1α in normal lung tissue was different from that found in inflamed lung tissue. In normal tissue, immunoreactivity to MIP-1α was confined to the bronchial epithelial layer. Significant staining of the alveoli was not observed in either the epithelial or stromal components (Fig. 1). SP-A was weakly expressed by type II lining cells. In the inflamed areas, macrophages exhibited strong MIP-1α immunoreactivity, although lymphocytes and other lung components did not significantly express MIP-1α. In addition, activated fibroblasts were strongly stained by MIP-1α antibody, while non-activated fibroblasts were not stained. Activated fibroblasts are larger than non-activated fibroblasts and have myofibroblastic features demonstrated by anti-α-smooth muscle actin immunostains. Hyperplastic alveolar type II cells were found in alveoli and were stained by MIP-1α (Fig. 2a) concomitant with an increase in SP-A immunostaining (Fig. 2b). However, the immunoreactivity in bronchial epithelium was not modified significantly.

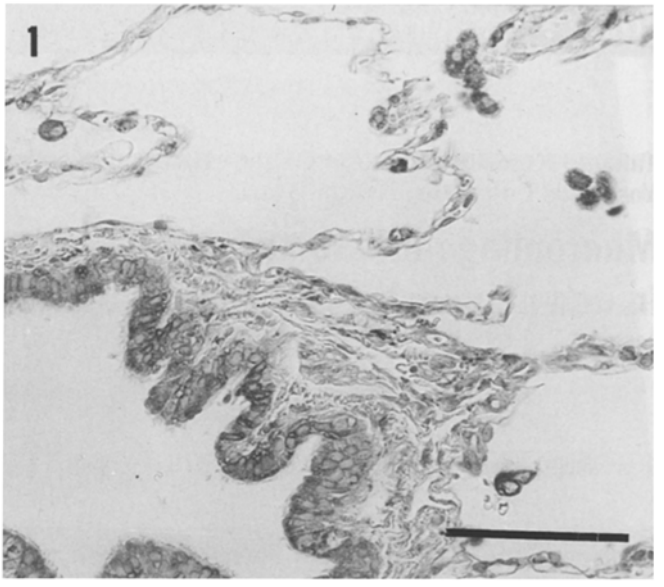

Fig. 1 Immunohistochemical study of normal lung tissue for macrophage inflammatory protein-1 alpha (MIP-1α). Positive staining was found in bronchial epithelial cells and a few alveolar macrophages. (*Bar*=100 μm, original magnification ×50)

Table 1 Immunohistochemical analysis of normal and inflamed lung tissue (*MIP-1α* macrophage inflammatory protein-1 alpha, *SP-A* surfactant protein A, *S* strongly reactive, *W* weakly reactive, *N* non-reactive)

Tissue type	Antibody	
	MIP-1α	SP-A
Normal lung tissue		
Bronchi and bronchioles		
Ciliated cells	S	N
Basal cells	W	N
Alveoli		
Type I cells	N	N
Type II cells	N	W
Fibroblasts	N	N
Macrophages	S	W
Inflamed lung tissue		
Bronchi and bronchioles		
Ciliated cells	S	N
Basal cells	W	N
Alveoli hyperplastic type II cells	S	S
Activated fibroblasts	S	N
Macrophages	S	W

Patient characteristic, tumour histology and differentiation, pathological stage (TNM) and imunhistochemical findings are summarized in Table 2. Twenty-two tumours were adenocarcinomas (Fig. 3), 8 were squamous cell carcinomas (Fig. 4) and 3 were small cell carcinomas. Twenty-three (70%) of the malignant tumours expressed MIP-1α; 19 (86%) adenocarcinomas, 4 (50%) squamous cell carcinomas. However, the 3 small cell carcinomas did not express MIP-1α. In adenocarcinomas, essentially all of the tumour cells were strongly reactive (+++), whereas in squamous cell carcinoma only a portion of

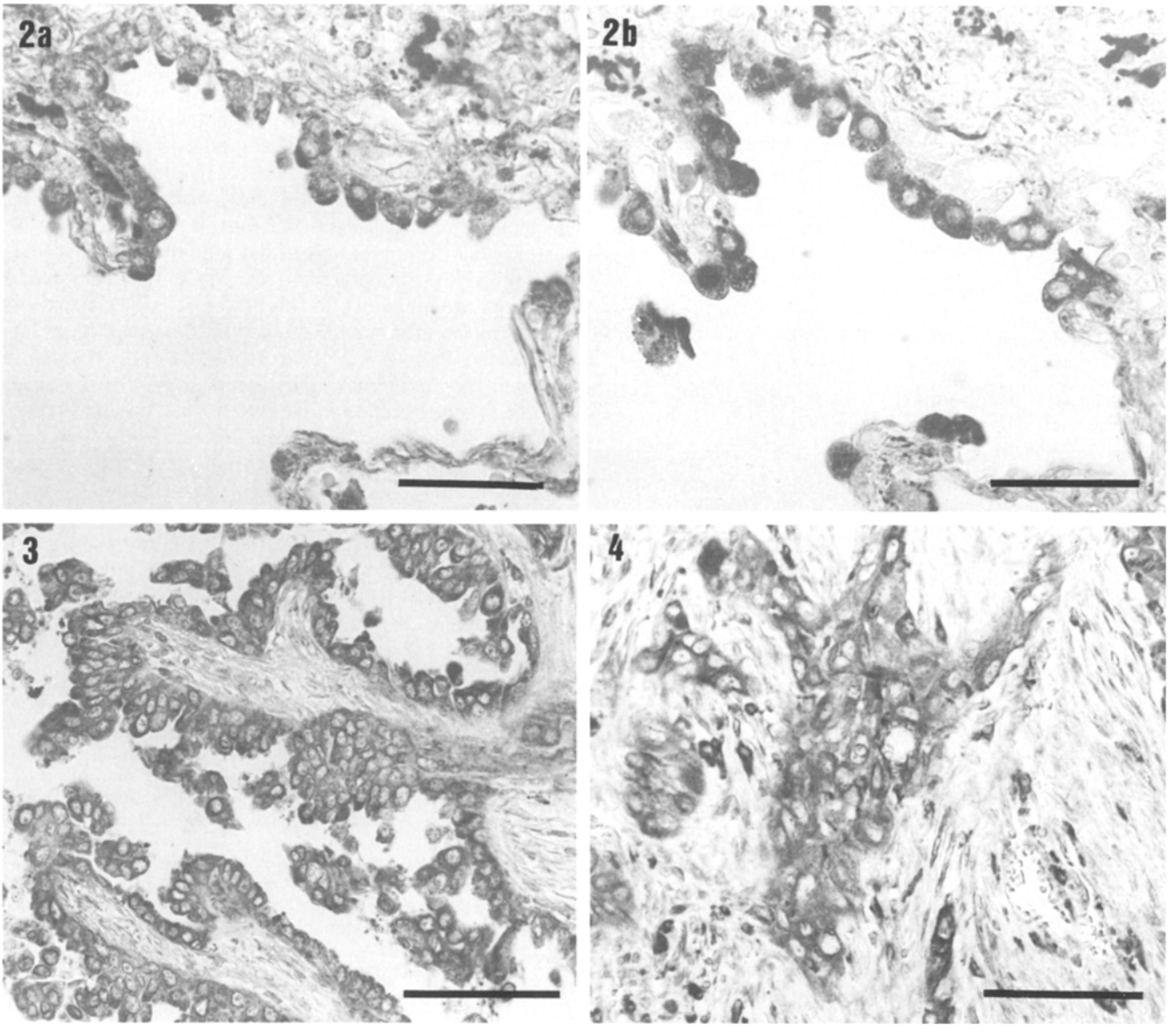

Fig. 2a, b Immunohistochemical study of inflamed lung tissue. Expression of MIP-1α (**a**) and surfactant protein-A (**b**) was found in hyperplastic alveolar type II cells. (*Bar*=40 μm, original magnification ×62.5)

Fig. 3 Strong immunoreactivity for MIP-1α in adenocarcinoma. (*Bar*=100 μm, original magnification ×50)

Fig. 4 Positive staining for MIP-1α was found in squamous cell carcinoma and interstitial cells such as macrophages and activated fibroblasts (*Bar*=100 μm, original magnification ×50)

the malignant cells were reactive (+). Interstitial macrophages and fibroblasts found in the interstitium of neoplastic tissue were MIP-1α immunoreactive (Fig. 4). The degree of macrophage accumulation and interstitial fibrosis was not significantly related to MIP-1α expression by neoplastic cells. SP-A-reactive cells were found in 8 (36%) of the adenocarcinomas, but in none of the squamous cell or small cell carcinomas. There was no significant correlation between the distribution of staining and staining intensity of MIP-1α and SP-A.

Discussion

In the lung tissue, bronchial epithelium, type II alveolar pneumocytes and fibroblasts have been demonstrated to synthesize several chemokines such as MCP-1 [6, 20, 26] and IL-8 [5, 12, 16, 19, 22, 23]. MIP-1α is also expressed by murine bronchial epithelial cells [18]. We also demonstrated MIP-1α expression in human bronchial epithelial cells including ciliated cells and basal cells by immunohistochemical analysis.

In inflammatory conditions, fibroblasts immunostained intensively for MIP-1α, concomitant with their activation. This finding is consistent with previous studies of inflammatory conditions such as sarcoidosis and fibrosing lung diseases [21]. Activated fibroblasts are larger than non-activated fibroblasts, and are also characterized by increased proliferative activity and collagen synthesis [4, 15]. It is often difficult to identify individual activated fibroblasts on routine light microscopy.

Table 2 Immunohistochemical analysis of lung cancer cells (Ad adenocarcinoma, *Sq* squamous cell carcinoma, *Sm* small cell carcinoma, *Mod* moderate)

Age/sex	Histology	Differentiation	Stage (ptNM)	MIP-1α	SP-A
72/M	Ad	Poorly	100	+++	–
74/M	Ad	Well	100	+++	+++
66/M	Ad	Well	100	+++	–
46/M	Ad	Well	100	+++	++
57/M	Ad	Well	100	+++	++
64/F	Ad	Well	100	++	–
65/M	Ad	Well	200	+++	++
71/M	Ad	Mod	200	+++	–
77/M	Ad	Well	200	+++	–
58/M	Ad	Mod	110	++	–
71/M	Ad	Well	220	+++	++
55/M	Ad	Poorly	220	+++	–
45/M	Ad	Poorly	300	+	–
68/M	Ad	Mod	310	++	–
63/M	Ad	Mod	400	–	–
54/M	Ad	Mod	400	+++	–
61/F	Ad	Well	410	+++	–
60/F	Ad	Well	420	+++	+
54/F	Ad	Mod	420	+++	–
67/F	Ad	Mod	420	+++	+++
58/M	Ad	Mod	420	–	–
72/M	Ad	Poorly	430	–	++
71/M	Sq	Well	200	–	–
69/M	Sq	Mod	200	–	–
71/M	Sq	Mod	200	+	–
72/M	Sq	Well	200	+	–
66/F	Sq	Well	210	–	–
52/M	Sq	Mod	210	+	–
67/M	Sq	Mod	220	+	–
80/M	Sq	Poorly	321	–	–
78/M	Sm		100	–	–
45/M	Sm		400	–	–

However, we were able to identify these activated fibroblasts by their smooth muscle actin expression which is consistent with their myofibroblastic phenotype [27].

MIP-1α expression has not been previously described in alveolar cells. However, our immunohistochemical studies revealed intense MIP-1α immunoreactivity in hyperplastic alveolar type II cells concomitant with an increase in SP-A immunoreactivity in these cells. Both hyperplastic alveolar type II cells and activated fibroblasts were distributed in areas of interstitial fibrosis or persistent inflammatory changes. Therefore, the environmental factors that affect hyperplastic alveolar type II cells and activated fibroblasts appear to be similar. In addition, environmental stimuli may upregulate the synthesis of MIP-1α by these two types of cells. Since MIP-1α synthesis by activated fibroblasts is upregulated by cytokines such as IL-1, interferon-gamma, and IL-10 [1], cytokine stimulation may also play an important role in MIP-1α expression by activated fibroblasts and alveolar type II cells in fibrosing or persistent inflammatory lesions.

Several cytokines such as IL-8 [3, 10] and IL-6 [8] are secreted by lung carcinoma cells, but the synthesis of MIP-1α by malignant cells has not been previously described. Using immunohistochemical techniques, we demonstrated frequent MIP-1α expression in adenocarcinoma and squamous cell carcinoma, but not in small cell carcinoma. The ratio of reactive cells in individual tumours was much higher in adenocarcinomas than in squamous cell carcinomas, and intensity of immunostaining was also stronger. The synthesis of this chemokine therefore appears to be most active in adenocarcinomas. As previously described, the distribution of MIP-1α in adenocarcinomas dose not correlate with either the histological differentiation of the tumour or the distribution of SP-A immunoreactivity. Since this chemokine regulates monohistiocytic migration, it may also be involved in the inflammatory reaction found within malignant tissue. However, MIP-1α is not the only chemokine secreted by carcinomas. The understanding of the exact role of this chemokine in carcinomas will require correlation between other cytokines derived from malignant cells and from benign mesenchymal components within the neoplasm.

Our findings suggest that activated alveolar type II cells and fibroblasts can participate in inflammatory cell recruitment via the production of MIP-1α. Additionally, tumour derived MIP-1α may affect the interactions between lung cancer and host inflammatory cells.

References

1. Christman JW, Blackwell TR, Cowan HB, Shepherd VL, Rinaldo JE (1992) Endotoxin induces the expression of macrophage inflammatory protein 1α mRNA by rat alveolar and bone marrow-derived macrophages. Am J Respir Cell Mol Biol 7:455–461
2. Fujiyama Y, Saitoh Y, Ide T, Sasaki T, Niwakawa M, Hodohara K, Bamba T, Tokiyoshi S, Okabe H, Hosoda S (1993) Cytokine LD78, a human homolog of mouse macrophage inflammatory protein 1-α immunoreactivity in the human intestinal tract. In: Tsuchiya M, Yodoi J, Hibi T, Miura S (eds) Current topics in mucosal immunology. Excerpta Medica, Amsterdam, pp 263–266
3. Hotta K, Hayashi K, Ishikawa J, Tagawa M, Hashimoto K, Mizuno S, Suzuki K (1990) Coding region structure of interleukin-8 gene of human lung giant cell carcinoma LU65C cells that produce LUCT/interleukin-8: homogeneity in interleukin-8 genes. Immunol Lett 24:165–170
4. Jordana M, Schulman J, McSherry C, Irving LB, Newhouse MT, Jordana G, Gauldie J (1988) Heterogenous proliferative characteristic of human adult lung fibroblasts and clonally derived fibroblasts from control and fibrotic tissue. Am Rev Respir Dis 137:579–584
5. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. Immunology 68:31–36
6. Larsen CG, Zachariae COG, Oppenheim JJ, Matsushima K (1989) Production of monocyte chemotactic and activating factor (MCAF) by human dermal fibroblasts in response to interleukin 1 or tumor necrosis factor. Biochem Biophys Res Commun 160:1403–1408
7. Lukacs NW, Chensue SW, Smith RE, Strieter RM, Warmington K, Wilke C, Kunkel SL (1994) Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α by inflammatory granuloma fibroblasts. Am J Pathol 144:711–718
8. Matsuguchi T, Okamura S, Kawasaki C, Shimoda K, Omori F, Hayashi S, Kimura N, Niho Y (1991) Constitutive production

of granulocyte colony-stimulating factor and interleukin-6 by a human lung cancer cell line, KSNY: gene amplification and increased mRNA stability. Eur J Haematol 47:128–133
9. Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ (1989) Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 169:1485–1490
10. Mizuno K, Sone S, Orino E, Mukaida N, Matsushima K, Ogura T (1994) Spontaneous production of interleukin-8 by human lung cancer cells and its augmentation by tumor necrosis factor alpha and interleukin-1 at protein and mRNA levels. Oncology 51:467–471
11. Mizutani Y, Nakajima T, Morinaga S, Gotoh M, Shimosato Y, Akino T, Suzuki A (1988) Immunohistochemical localization of pulmonary surfactant apoproteins in various lung tumors. Cancer 61:532–537
12. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG (1992) Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J Clin Invest 89:1478–1484
13. Oh KO, Zhou Z, Kim KK, Samanta H, Fraser M, Kim YJ, Broxmeyer HE, Kwon BS (1991) Identification of cell surface receptors for murine macrophage inflammatory protein-1α. J Immunol 147:2978–2983
14. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K (1991) Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu Rev Immunol 9:617–648
15. Phan SH, Varani J, Smith D (1985) Rat lung fibroblasts collagen metabolism in bleomycin-induced pulmonary fibrosis. J Clin Invest 76:241–247
16. Rolfe MW, Kunkel SL, Standiford TJ, Chensue SW, Allen RM, Evanoff HL, Phan SH, Strieter RM (1991) Pulmonary fibroblast expression of interleukin-8: a model for alveolar macrophage-derived cytokine networking. Am J Respir Cell Mol Biol 5:493–501
17. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796
18. Smith RE, Strieter RM, Phan SH, Lukacs NW, Huffnagle GB, Wilke CA, Burdick MD, Lincoln P, Evanoff H, Kunkel SL (1994) Production and function of murine macrophage inflammatory protein-1α in bleomycin-induced lung injury. J Immunol 153:4704–4714
19. Standiford TJ, Kunkel SL, Basha MA, Chensue SW, Lynch JP III, Toews GB, Westwick J, Strieter RM (1990) Interleukin-8 gene expression by a pulmonary epithelial cell line. J Clin Invest 86:1945–1953
20. Standiford TJ, Kunkel SL, Phan SH, Rollins BJ, Strieter RM (1991) Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like cells. J Biol Chem 266:9912-9918
21. Standiford TJ, Rolfe MW, Kunkel SL, Lynch JP III, Burdick MD, Gilbert AR, Orringer MB, Whyte RI, Strieter RM (1993) Macrophage inflammatory protein-1α expression in interstitial lung disease. J Immunol 151:2852–2863
22. Strieter RM, Phan SH, Showell HJ, Remick DG, Lynch JP, Genord M, Raiford C, Eskandari M, Marks RM, Kunkel Sl (1989) Monokine-induce neutrophil chemotactic factor gene expression in human fibroblasts. J Biol Chem 264:10621–10626
23. Vittori E, Sciacca F, Colotta F, Mantovani A, Mattoli S (1992) Protective effect of nedocromil sodium on the interleukin-1-induced production of interleukin-8 in human bronchial epithelial cells. J Allergy Clin Immunol 90:76–84
24. Wolpe SD, Cerami A (1989) Macrophage inflammatory protein 1 and 2: members of a novel superfamily of cytokines. FASEB J 3:2565–2573
25. Wolpe SD, Davatelis G, Sherry B, Beutler B, Hesse DG, Nguyen HT, Moldawer L, Nathan CF, Lowry SF, Cerami A (1988) Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med 167:570–581
26. Yoshimura T, Leonard EJ (1990) Secretion by human fibroblasts of monocyte chemoattractant protein-1, the product of gene JE. J Immunol 144:2377–2383