Clin. exp. Immunol. (1992) **88**, 181–187

Characterization of IL-1 inhibitory factor released from human alveolar macrophages as IL-1 receptor antagonist

M. TAKEUCHI, S. NAGAI, H. NAKADA*, H. AUNG, N. SATAKE, H. KANESHIMA & T. IZUMI
*Chest Disease Research Institute, Kyoto University, Kyoto, Japan, *Department of Biotechnology, Faculty of Engineering, Kyoto Sangyo University, Kyoto, Japan*

(Accepted for publication 18 November 1991)

SUMMARY

IL-1 possesses pleiotropic properties on various cells and its activity may be stringently regulated in several ways. We have previously reported that both IL-1 and its inhibitory factor are concomitantly released from alveolar macrophages in both healthy subjects and patients with chronic inflammatory lung diseases. An increase in IL-1 activities and a decrease in inhibitory activities are characteristics found in both healthy smokers and patients with interstitial lung diseases. In this study, we further examined the biological properties of IL-1 inhibitory factor. The inhibitor exhibited a dose-dependent specific inhibition of an augmentation by IL-1 of PHA-induced murine thymocyte proliferation, while no inhibition of the augmentation by IL-2, IL-4, IL-6, or tumour necrosis factor (TNF) was found. ^{125}I-labelled IL-1α binding on PHA-stimulated murine thymocytes revealed two types of IL-1 binding sites, 44 sites/cell with a K_d of $2{\cdot}7\times10^{-10}$ M and 230 sites/cell with a K_d of $2{\cdot}5\times10^{-9}$ M. Alveolar macrophage culture supernatants blocked the binding of labelled IL-1 to the IL-1 receptor in a dose-dependent fashion. Scatchard plot analysis revealed that the inhibitory factor in the supernatants blocked the binding competitively. These results indicate that alveolar macrophages produce a specific IL-1 inhibitory factor, functioning as an IL-1 receptor antagonist.

Keywords human alveolar macrophages IL-1 IL-1 inhibitory factor IL-1 receptor IL-1 receptor antagonist

INTRODUCTION

Alveolar macrophages (AM) are involved in various inflammatory processes of the lung in both healthy and diseased states. Various cytokines, mediators, proteases and oxygen metabolites released from AM play important roles in these processes, acting on various target cells.

IL-1 exhibits pleiotropic activities in inflammatory sites: in the immune response through its enhancing action on PHA-stimulated thymocyte proliferation, in the inflammatory reaction through the stimulation of prostaglandin E2(PGE2) production [1], in destructive processes through the stimulation of collagenase production of fibroblasts [2], or through the stimulation of proliferation of fibroblasts [3]. In lungs, AM can release IL-1 constitutively, probably due to various stimuli given exogenously or endogenously [4]. However, its activity is limited in lungs, compared with that in blood [5]. Our previous report demonstrated the presence of IL-1 inhibitory activities in culture supernatants of AM in both healthy and interstitial lung diseases [6]. The inhibitory activities were decreased in healthy smokers and diseased groups with low-grade inflammation of the lung, compared to those in healthy nonsmokers [7]. Considering these results, an imbalance between the release of IL-1 and that of the IL-1 inhibitor may evoke an inflammatory state in the lungs.

During the last few years, many inhibitors of IL-1 activities have been reported [8]. The sources of such inhibitors are mainly urine [9] and monocyte/monocyte cell lines [10]. There are various sites on which IL-1 inhibitors act. Recently, some reports showed that IL-1 inhibitor derived from monocytes can act specifically on the IL-1 receptor as an antagonist [11–12]. On the other hand, a specific IL-1 inhibitor from human AM has been less well characterized [13–15]. Therefore, we tried to examine our IL-1 inhibitor released from human AM, in terms of its specificity and the site of action.

Correspondence: S. Nagai, Second Department of Medicine, Chest Disease Research Institute, Kyoto University, 53 Kawaramachi, Shogo-in, Sakyo-ku, Kyoto 606, Japan.

MATERIALS AND METHODS

Isolation of human alveolar macrophages

Alveolar macrophages were obtained from 12 healthy non-smokers using bronchoalveolar lavage (BAL) techniques, as previously described [16]. Recovered BAL fluid (BALF) was filtered through gauze and the cells were collected by centrifuga-

tion at 400 *g* for 10 min at 4°C. The BALF AM fractions were further purified by rosetting with sialidase-treated sheep erythrocytes. After Ficoll-Paque gradient centrifugation, the non-rosetting cell fraction was collected, washed and resuspended in RPMI 1640 containing 15% FCS at 1×10^6 cells/ml. After the purification of non-rosetting cells by plastic adhesion, the adherent cells contained AM purified up to 95% purity as determined by the non-specific esterase staining technique.

Preparation of AM culture supernatants

The AM was washed and resuspended in RPMI 1640 supplemented with 15% FCS at 1×10^6 cells/ml. One millilitre of cell suspension was placed into each well of 24-well tissue culture plates and were incubated with 10 μg/ml lipopolysaccharide B(LPS B *E. coli* 055:B5) for 24 h at 37°C in humidified air containing 5% CO_2. After 24 h, AM culture supernatants were harvested and stored at −70°C until later use.

Bioassays and immunoassays of IL-1

The IL-1 activity in the supernatants was measured by a bioassay in which a proliferative response of murine thymocytes (4- to 6-week-old C3H/HeJ mice) to 5 μg/ml of PHA (Wellcome, Dartford, UK) was used as an index, as previously described [6].

The amounts of immunoreactive IL-1 in the AM culture supernatants were determined by radioimmunoassay (IL-1 RIA kit: Cistron, Pine Brook, NJ).

Bioassay of IL-1 inhibitory activity

IL-1 inhibitory activities in AM culture supernatants were measured by suppression of a proliferative response of murine thymocytes to a constant amount of human recombinant IL-1α (Dainippon Pharmaceutical Co., Osaka, Japan), as previously described, with a slight modification [6,7].

Murine thymocytes prepared from 4- to 6-week-old C3H/HeJ mice were resuspended in RPMI-1640 supplemented with 10% FCS, 500 U/ml penicillin, and 5·0 μg/ml gentamicin. The cells (1×10^6 cells/ml) were cultured in 96-well flat-bottomed microplates (Corning 25860, Corning Glass Works, Corning, NY), for 72 h at 37°C in 5% CO_2 in the presence of PHA (5 μg/ml) and IL-1 (200 pg/ml) and serially diluted AM culture supernatants. Cultured thymocytes were pulsed with 1·85 MBq/well of ^{3}H-thymidine (NEN, Boston, MA) for the final 15 h of the incubation. Cells were harvested onto glass filter-paper discs and the radioactivity was measured by a liquid scintillation counter. The data were expressed as mean counts of triplicates. The IL-1 inhibitory activity (%) in the supernatants was calculated as follows: IL-1 inhibitory activity (%)

$$\left\{1 - \frac{\text{(IL-1 + AM sup.) (ct/min)} - \text{(medium) (ct/min)}}{\text{(IL-1) (ct/min)} - \text{(medium) (ct/min)}}\right\} \times 100.$$

Inhibitory activity towards various cytokines other than IL-1 was assayed with PHA stimulated thymocytes (1×10^6 cells/well). Namely, the cells were cultured for 72 h in the presence of 1000 U/ml of human IL-2 (Pharmacia ENI Diagnostic, Silver Springs, Maryland), 250 U/ml of human IL-4, 500 U/ml of human IL-6, and 500 U/ml of human TNF, respectively (IL-4, IL-6, and TNF were purchased from Genzyme, Boston, MA). The optimal concentration was chosen in these experiments, which in preliminary experiments had shown a substantial proliferative response of murine thymocytes. Simultaneously, we compared the effect of AM culture supernatants (four-fold dilution) on the above responses to each cytokine with IL-1. After 72 h of culture, ^{3}H-thymidine incorporated into cells was measured by the same procedure as described above.

^{125}I-IL-1α binding assay

^{125}I-IL-1α binding to PHA-stimulated thymocytes was measured as previously described [17]. Briefly, murine thymocytes (1×10^6 cells/ml) were cultured for 3 days in RPMI 1640 supplemented with 5% FCS, containing PHA (5 μg/ml) as a stimulatory agent. After 3 days, 100 μl of the PHA-stimulated thymocytes suspension of 2×10^7 cells/ml were incubated in the presence (large excess) or absence of unlabelled IL-1α in RPMI 1640 medium supplemented with 1% BSA, and 20 mM HEPES buffer (pH 7.2), for 1 h at 4°C. Then, various amounts of ^{125}I IL-1α were added to a total volume of 130 μl and incubated for 2 h at 4°C with gentle agitation. The non-specific binding of ^{125}I IL-1α was measured in the presence of a 200-fold excess of unlabelled IL-1α. At the end of the incubation, 130 μl aliquots of the incubation mixture were centrifuged on a phthalate oil gradient to separate ^{125}I-IL-1α from cell-bound ^{125}I-IL-1α. The tubes were frozen in a dry ice-ethanol batch, cut, and radioactivities in each of the cut parts were counted by a gamma counter. A Scatchard plot analysis of the saturation curve was performed as previously described [18].

The capacity of PHA-stimulated murine thymocytes to bind ^{125}I IL-1α was compared with those in the presence of various dilutions of AM culture supernatants.

Treatment of AM culture supernatants with anti-human IL-1 antibodies

Anti-human IL-1α and β antibodies (Cistron, Pine Brook, NJ) conjugated with protein A Sepharose (Pharmacia, Uppsala, Sweden) were prepared according to the method of Schneider *et al.* [19]. AM culture supernatants (400 μl of four-fold dilution) were incubated with the antibody-protein A-Sepharose for 3 h at 4°C under constant agitation and then centrifuged. The supernatants obtained were assayed as described earlier.

Chemical cross-linking of thymocytes with bound ^{125}I-IL-1α

PHA-stimulated thymocytes (1×10^7 cells) were incubated in 200 μl of RPMI 1640 contained 1% BSA, and ^{125}I IL-1α (5×10^{-10} M) in the presence or absence of AM supernatants for 3 h at 4°C. The cells were then washed twice with chilled PBS and suspended in 100 μl PBS. Disuccinimidyl suberate (DSS) in dimethyl sulphoxide (DMSO) was added to give a final concentration of 1 mg/ml, and the mixture was incubated for 1 h at 4°C. Subsequently, cells were washed twice with PBS, and were lysed by suspending them in 50 μl of PBS containing 1% Triton X-100 and 2 mM phenylmethylsulphonyl fluoride (PMSF) and incubated for 15 min on ice. The suspension was centrifuged at 8000 *g* for 5 min. Forty microlitres of the cell lysate were subjected to SDS-polyacrylamide gel (8·5%) electrophoresis according to Laemmli [20], and the gels were stained with Coomassie Brilliant Blue. The dried gel was exposed to a XAR-5 film for 3 days at 20°C to obtain an autoradiograph by an image analyser (Fuji Photo Film, Tokyo, Japan). The control experiment was carried out in the presence of a 200-fold excess of unlabelled IL-1α.

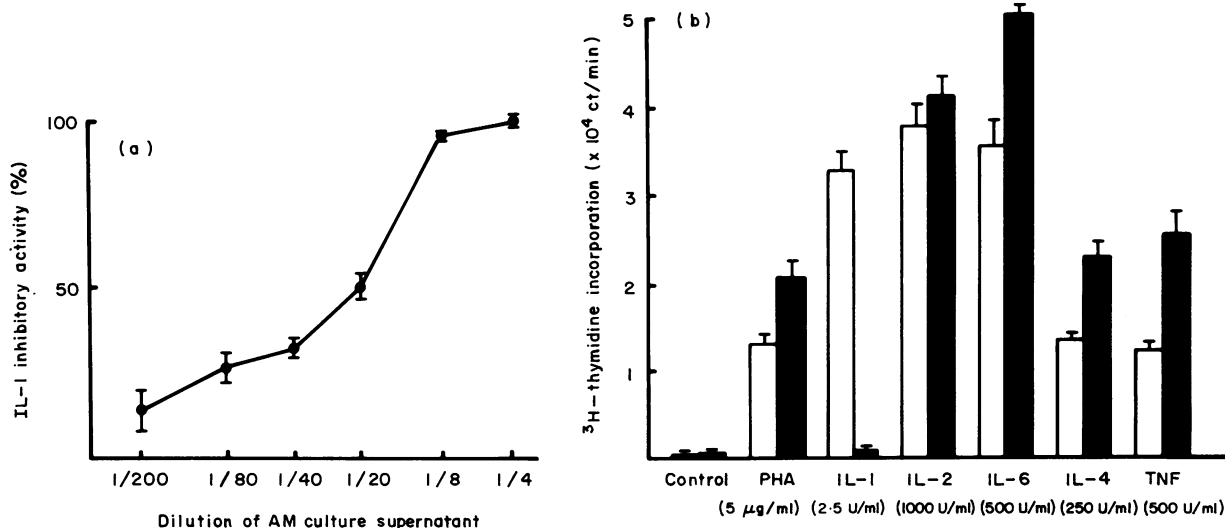

Fig. 1. IL-1 inhibitory activity in AM culture supernatants and its specificity on IL-1 induced response. (a) IL-1 inhibitory activity in AM culture supernatants. Murine thymocytes (1×10^7 cells/ml) were cultured with PHA (5 μg/ml), IL-1 (200 pg/ml) and AM culture supernatants diluted serially, as indicated in the figure. (b) Specificity of the inhibitory activity to IL-1 induce responses. Thymocyte proliferation was measured by ^{3}H-thymidine incorporation in response to PHA, PHA and IL-1, PHA and IL-2, PHA and IL-4, PHA and IL-6, and PHA and TNF in the absence (□) or presence (■) of AM culture supernatants (four-fold dilution).

Gel filtration of AM culture supernatants

A concentrated AM culture supernatant (5 ml) was applied to a column of Sephacryl S-200 (1·6 × 95 cm, Pharmacia, Uppsala, Sweden), equilibrated at 4°C with 50 mM Tris-HCl buffer (pH 7·5), containing 150 mM NaCl. A fraction of 6 ml was collected and IL-1 inhibition was assayed. Calibration was performed by using blue dextran (mol. wt 2000 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), chymotrypsinogen A (25 kD), and ribonuclease A (13·7 kD).

We used this fraction for some experiments, but in the binding experiments we used the whole supernatants, because of the shortage of the amounts available to the experiments.

RESULTS

IL-1 inhibitory activities in AM culture supernatants from healthy subjects

Pooled AM culture supernatants of healthy subjects were serially diluted with culture medium, and their IL-1 inhibitory activities were determined by a bioassay. A dose-dependent inhibition of the proliferation of murine thymocytes was detected (Fig. 1a). The inhibition was 12–98%, in the range from 200-fold to 4-fold dilutions of AM culture supernatants. Essentially, all the cells were viable during the incubation at any dilution of the culture supernatants.

The specificity of the inhibitory activity in AM culture supernatants

We examined the specificity of the above inhibitory activities. Instead of IL-1, we used IL-2, IL-4, IL-6, or TNF in our inhibitory assay. IL-2 and IL-6 showed an enhancement of the proliferation similar to IL-1, whereas other cytokines had a lower proliferative activity. An inhibitory activity in AM culture supernatants was detected only in the presence of IL-1 (Fig. 1b).

Binding of ^{125}I-IL-1α to PHA-stimulated murine thymocytes

^{125}I-IL-1α of various concentrations was incubated for 2 h at 4°C with murine thymocytes which had been cultured for 3 days with PHA. Cell-bound radioactivities were plotted versus the concentration of ^{125}I-IL-1α. The specific binding was calculated by subtracting the nonspecific binding from the total one (Fig. 2a) The specific binding curve reached a plateau at a ligand concentration of 400 pM. A Scatchard plot analysis of the saturation curve revealed two types of IL-1 binding sites on PHA-stimulated murine thymocytes (Fig. 2b). One binding site had 44 sites per cell with a K_d (dissociation constant) value of $2{\cdot}7 \times 10^{-10}$ M, and the other 230 sites per cell and a K_d of $2{\cdot}5 \times 10^{-9}$ M.

Inhibition of ^{125}I-IL-1α binding on thymocytes by AM culture supernatants

Pretreatment of PHA-stimulated murine thymocytes with AM culture supernatants inhibited the binding of ^{125}I-IL-1α to thymocytes. This inhibition was dose-dependent in a range of two- to 25-fold dilutions (Fig. 3a). Inhibition of the binding of ^{125}I-IL-1α to thymocytes with fixed dilutions of supernatants, and varying concentrations of the ligand is shown in Fig. 4a. A Scatchard plot analysis of this saturation curve revealed that the inhibitor in the supernatants blocked the binding competitively (Fig. 4b).

After treatment of the supernatants, in which the IL-1 activity was present, with excessive amounts of anti-IL-1 antibody, the intensity of the inhibition was unchanged (Fig. 3b).

Identification of the receptor–ligand complex by affinity cross linking and the inhibition of complex formation by AM culture supernatants

The competitive inhibition of the binding of ^{125}I-IL-1α to PHA-stimulated thymocytes by AM culture supernatants suggested that the inhibitory factor may act as an antagonist of IL-1 by

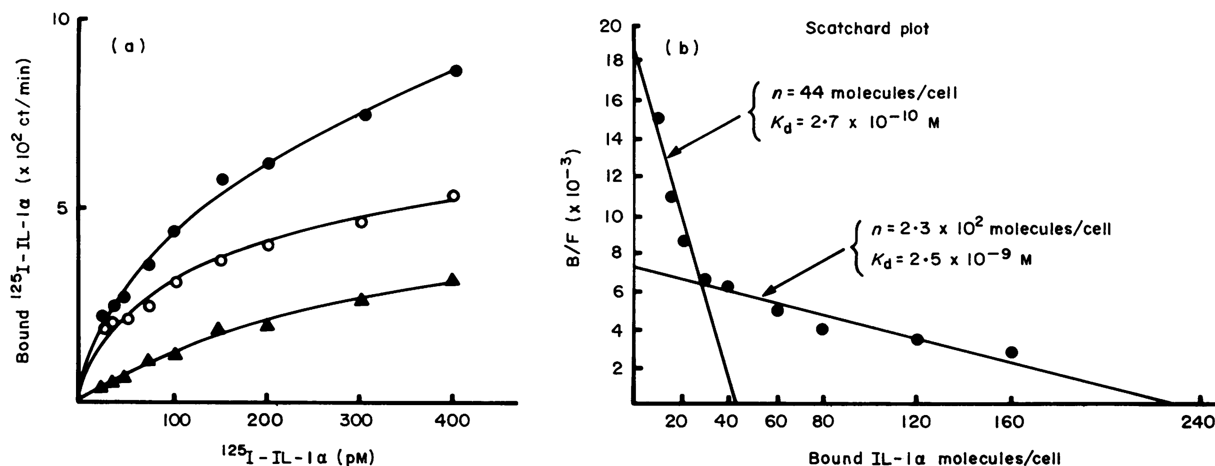

Fig. 2. Binding of ^{125}I-IL-1α to PHA-stimulated murine thymocytes and Scatchard plot analysis of the saturation curve. (a) PHA-stimulated thymocytes were incubated at 4°C in the presence of various amounts of ^{125}I-IL-1α for 2 h. Nonspecific binding was estimated in the presence of a 200-fold excess of unlabelled IL-1α. (●) Total binding activity; (○) specific binding (total-nonspecific); (▲) nonspecific binding activity. (b) Scatchard plot analysis of the saturation curve shown in (a).

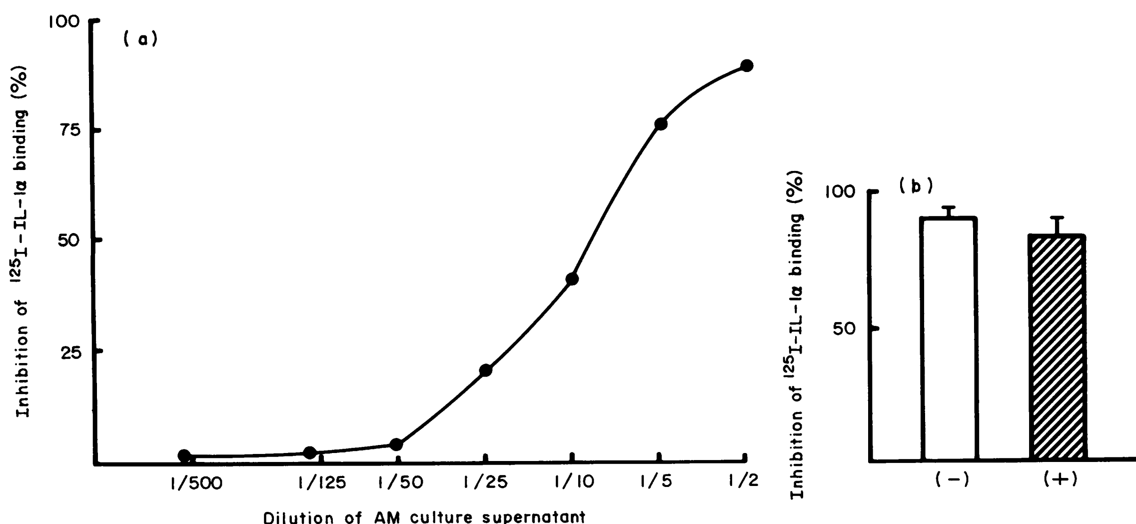

Fig. 3. Inhibition of ^{125}I-IL-1α binding of PHA-stimulated murine thymocytes by AM culture supernatants. (a) PHA-stimulated thymocytes were preincubated for 1 h at 4°C with AM culture supernatants diluted (*4-fold*) as indicated in the figure, and then ^{125}I-IL-1α was added to a final concentration of 50 pM and incubated for 2 h at 4°C. Nonspecific binding was estimated in the presence of a 200-fold excess of unlabelled IL-1α. (b) Effect of anti-IL-1α and β antibodies to the inhibitory activity in AM culture supernatants on the ^{125}I-IL-1α binding to PHA-stimulated thymocytes. PHA-stimulated thymocytes were preincubated for 1 h at 4°C with a 4-fold dilution of AM culture supernatant, treated with (+) or without (−) anti-IL-1 antibody. Then, ^{125}I-IL-1α was added to a final concentration of 50 pM and incubated for 2 h at 4°C. Inhibition of the binding (%) was expressed as follows:

$$\left\{\frac{\text{AM sup (ct/min)} - \text{nonsp. binding (ct/min)}}{\text{total binding (ct/min)} - \text{nonsp. binding (ct/min)}}\right\} \times 100.$$

binding to its receptor. Therefore, we tried to demonstrate that the receptor–ligand complex could be identified and the formation of this complex is inhibited by pretreating thymocytes with the antagonist (supernatants).

After the receptor–ligand complex had been bound covalently by a cross-linking agent, the cell lysate was analysed by SDS-PAGE. Two radioactive bands were detected at the positions of about 80 and 100 kDa. The two radioactive bands were not detected in the presence of excessive amounts of unlabelled IL-1α, or of AM culture supernatants (Fig. 5). From these results it was deduced that the 80 and 100 kD bands represented a complex between ^{125}I-IL-1α and the IL-1 receptor on thymocytes, and that the IL-1 receptor antagonist in the supernatants completely blocked the complex formation.

Estimation of molecular size of the inhibitor in AM culture supernatants by gel filtration on Sephacryl S-200

Concentrated and dialysed AM culture supernatants were subjected to Sephacryl S-200 gel filtration. Collected fractions were examined for IL-1 by RIA and for IL-1 inhibitory activity

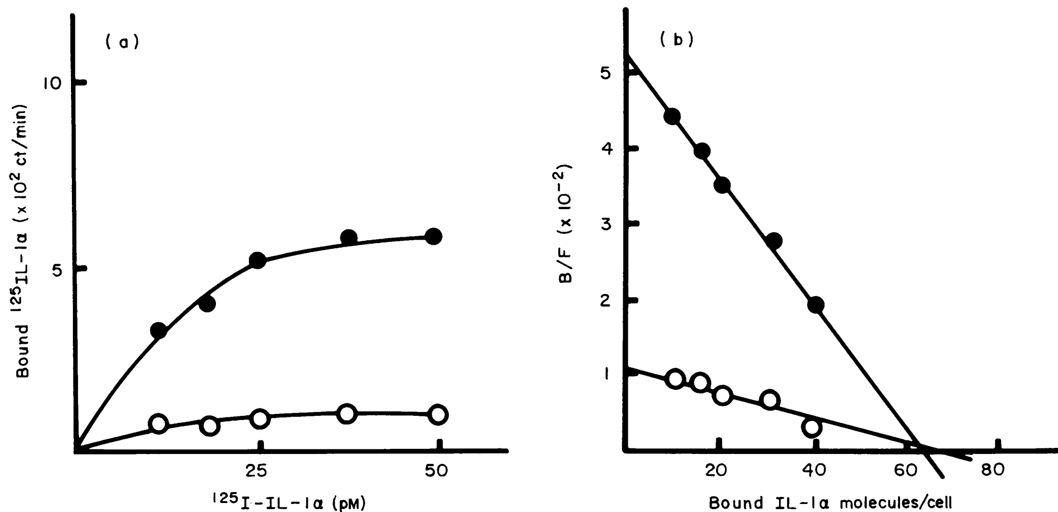

Fig. 4. Inhibition of ^{125}I-IL-1α binding to PHA-stimulated thymocytes by AM culture supernatants. (a) PHA-stimulated thymocytes were preincubated for 1 h in the presence (○) or absence (●) of AM culture supernatants, and then various amounts of ^{125}I-IL-1α were added and incubated for 2 h at 4°C. Nonspecific binding was estimated in the presence of a 200-fold excess of unlabelled IL-1α. (b) Scatchard plot analysis of both saturation curves in the presence or absence of AM culture supernatants shown in (a).

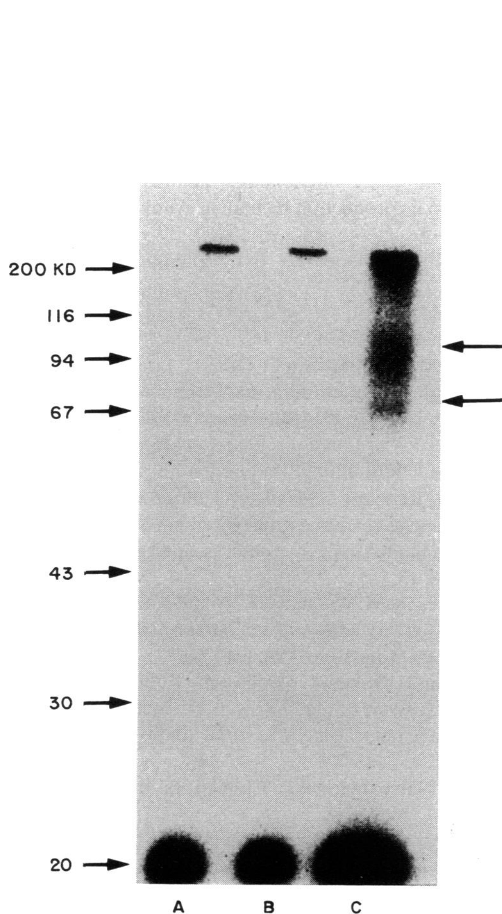

Fig. 5. Inhibition of ^{125}I-IL-1α binding to IL-1 receptor by AM culture supernatants. PHA-stimulated thymocytes were incubated with ^{125}I-IL-1α in the presence (lane B) or absence (lane C) of AM culture supernatants. A control sample was included which was cultured in the presence of a 200-fold excess of unlabelled IL-1α (lane A). After extensive washing, ^{125}I-IL-1α and its receptor were cross-linked and the cell lysate was subjected to SDS–PAGE followed by autoradiography.

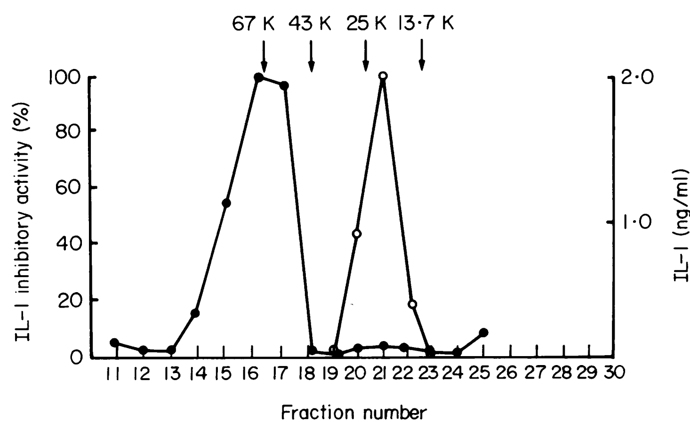

Fig. 6. Gel filtration on Sephacryl S-200 column of AM culture supernatants. Concentrated AM culture supernatants were fractionated on Sephacryl S-200. IL-1 inhibitory activity (●) was assayed by an IL-1 inhibitory bioassay, in which the serial suppression of AM culture supernatants to thymocyte proliferation in the response to standard IL-1 was examined. Immunoreactive IL-1 (○) was determined by radioimmunoassay.

by a bioassay (Fig. 6). This procedure could completely separate IL-1 inhibitory activity from IL-1. IL-1 inhibitory activities were mainly concentrated into two fractions (16 and 17). The molecular size was nearly the same as that of bovine serum albumin (67–71 kD). Due to the shortage of the amounts available, the whole supernatant was used for most of the binding experiments.

DISCUSSION

In our previous reports, an increase in IL-1 and a decrease in IL-1 inhibitory activities were demonstrated with the supernatants of alveolar macrophages from healthy long-term smokers, and patients with interstitial lung diseases, compared to healthy non-smokers [6–7]. These alterations may contribute

to a development of chronic low grade inflammation in the lung, due to chronic smoking, and interstitial lung diseases. We have already reported that the inhibitory activity in AM culture supernatants inhibit the stimulatory activity of IL-1 on both murine thymocytes and human fibroblasts [7]. However, IL-1 belongs to a group of cytokines with overlapping biological properties. IL-1, TNF, and IL-6 share the abilities to stimulate T and B cells, and to augment cell proliferation. Therefore, we investigated the specificity of the inhibitory activity on biological responses induced by various cytokines. The results showed that this inhibitory activity in the supernatants blocked the murine thymocyte proliferative response only in the presence of IL-1, but not in the presence of IL-2, IL-4, IL-6, or TNF.

IL-1 is capable of exerting pleiotropic effects on various target cells in both healthy and diseased states. It is important to examine how the IL-1 inhibitory factor blocks the IL-1-induced phenomenon, from the viewpoint of a therapeutic strategy.

As previously demonstrated, this factor is neither PGE_2 nor an anti-IL-1 antibody [21]. Therefore, we examined whether this factor is an IL-1 receptor antagonist or not. The binding of ^{125}I-IL-1α to PHA-stimulated murine thymocytes was competitively blocked by AM culture supernatants, indicating that the factor can be identified as a receptor antagonist.

As to the IL-1 receptor on murine thymocytes, a significant amount of the receptor could be detected only after PHA stimulation, as shown by several studies including ours (data not shown) and of the one by Dowers *et al.* [17]. Our results showed two types of receptors with different numbers of binding sites and K_d values. In general, the IL-1 receptors have been classified into IL-1 receptor type I (IL-1RtI), type II (IL-1RtII), and minor putative receptors, as revealed by cross-linking methods [22]. On T cells and fibroblasts, IL-1RtI (80 kD) was consistently detected whereas B cell lines possess IL-1RtII (68 kD). IL-1RtII differs from IL-1RtI in binding affinities, on and off rates, regulation of its surface expression, and the type of signals transduced [23]. It remains to be elucidated whether the two types of IL-1 binding sites on PHA-stimulated murine thymocytes correspond to IL-1RtI and IL-1RtII. The small differences in molecular sizes between the two proteins we found and the reported sizes for the two IL-1 receptors may reflect a different degree of glycosylation [24].

Specific IL-1 receptor antagonists have already been reported by several groups from various sources: human monocytes [12], culture supernatants of the U937 monocytic cell line [25], supernatants of the THP-1 human monocytic cell line [10], urine from febrile patients [26], and alveolar macrophage culture supernatants [27]. Some of them show quite similar molecular sizes (18–25 kD), but others are different. The molecular size of our IL-1 receptor antagonist was about 71 kD. Besides, at the position of 20–25 kD, no inhibitory activity was detected. Therefore, there is a possibility that we found a unique IL-1 receptor antagonist.

The reported IL-1 receptor antagonist (IL-1ra) blocks the IL-1 activity *in vitro* and *in vivo*. *In vitro*, the IL-1ra appears to occupy the IL-1RtI on T cells and fibroblasts with nearly the same affinity as that of IL-1 but without a demonstrable agonist activity [12]. Whether the human IL-1ra binds to both the murine and human IL-1 receptor remains to be investigated. It has been reported that the human IL-1ra did not block IL-1 binding to the murine IL-1RtII, but did block the binding of IL-1 to human cells bearing the IL-1RtII such as neutrophils and B cells [22]. In this report, it is pointed out that the optimal dose which causes effective blocking is different depending on the species. Thus, the human IL-1ra blocks the binding of IL-1 to murine T cells (IL-1RtI) at nearly equimolar concentrations, but a 10-fold to 50-fold molar excess of IL-1ra is required to block the binding of IL-1 to human IL-1RtII-bearing cells.

A therapeutic effect of IL-1ra is currently under investigation in various animal models of several diseases. IL-1ra reduces for instance the mortality from septic shock or severe pneumonia [28,29]. However, there are no reports whether IL-1ra is effective as a therapeutic agent for chronic inflammatory diseases, such as pulmonary fibrosis. It should be noted that induction of fibroblast and smooth muscle proliferation by IL-1 *in vitro* is often difficult to observe when cyclo-oxygenase inhibitors are present [30]. Further purification of our IL-1 antagonist and development of a sensitive assay to evaluate the blocking capacity of this IL-1ra on IL-1-induced fibroblast activation are needed.

ACKNOWLEDGMENTS

We thank Professor I. Yamashina for advising and reviewing our study; Dr F. Matsuda for performing an autoradiographical procedure; Mrs F. Tanioka for her technical assistance; Mrs and Mr Germeraad for checking linguistic problems with the manuscript.

REFERENCES

1 Dayer JM, Rochemonteix BG, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin-1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J Clin Invest 1986; **77**:645–8.
2 Balavoine JF, Rochemonteix BG, Williamson K, Seckinger P, Cruchaud A, Dayer JM. Prostaglandin E2 and collagenase production by fibroblasts and synovial cells is regulated by urine-derived human interleukin 1 inhibitor. J Clin Invest 1986; **78**:1120–4.
3 Schmidt JA, Mizel SB, Cohen D, Green I. Interleukin-1, a potential regulator of fibroblast proliferation. J Immunol 1982; **128**:2177–82.
4 Koretzky GA, Elias JA, Kay SL, Rossman, MD, Nowell PC, Daniele RP. Spontaneous production of interleukin 1 by human alveolar macrophages. Clin Immunol Immunopathol 1983; **29**:443–50.
5 Wewers MD, Rennard SI, Hance AJ, Bitterman PB, Crystal RG. Normal human alveolar macrophages obtained by bronchoalveolar lavage have a limited capacity to release interleukin-1. J Clin Invest 1984; **74**:2208–18.
6 Nagai S, Takeuchi M, Watanabe K, Aung H, Izumi, T. Smoking and interleukin-1 activity released from human alveolar macrophages in healthy subjects. Chest 1988; **94**:694–700.
7 Nagai S, Aung H, Takeuchi M, Kusume K, Izumi T. IL-1 and IL-1 inhibitory activity in the culture supernatants of alveolar macrophages from patients with interstitial lung diseases. Chest 1991; **99**:674–80.
8 Larrick JW. Naive interleukin 1 inhibitors. Immunol Today 1989; **10**:61–6.
9 Liao Z, Grimshaw RS, Rosenstreich DL. Identification of a specific interleukin-1 inhibitor in the urine of febrile patients. J Exp Med 1984; **159**:126–36.
10 Bienkowski MJ, Eessalu TE, Berger AE, *et al.* Purification and characterization of interleukin 1 receptor level antagonist protein from THP-1 cells. J Biol Chem 1990; **265**:14505–11.
11 Arend WP, Joslin FG, Thompson RG, Hannum CH. An IL-1 inhibitor from human monocytes. J Immunol 1989; **143**:1851–8.
12 Hannum CH, Wilcox CJ, Arend WP, *et al.* Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990; **343**:336–40.

13 Roberts NJ, Prill AH, Mann TN. Interleukin-1 and interleukin-1 inhibitor production by human macrophages exposed to influenza virus or respiratory syncytial virus. Respiratory syncytial virus is a potent inducer of inhibitor activity. J Exp Med 1986; **163**:511–9.
14 Gosset P, Lassalle P, Tonnel AB. Production of an interleukin-1 inhibitory factor by human alveolar macrophages from normal and allergic asthmatic patients. Am Rev Respir Dis 1988; **138**:40–6.
15 Rochemonteix BG, Dayer JM, Junod AF. Fibroblast alveolar cell interactions in sarcoidosis and idiopathic pulmonary fibrosis: evidence for stimulatory and inhibitory cytokine production by alveolar cells. Eur Respir J 1990; 3:653–64.
16 Nagai S, Fujimura N, Hirata T, Izumi T. Differentiation between idiopathic pulmonary fibrosis and interstitial pneumonia associated with collagen vascular disease by comparison of the ratio of $OKT4^+$ cells and $OKT8^+$ cells in BALF T lymphocytes. Eur J Respir Dis 1985; **67**:1–9.
17 Dower SK, Kronheim SR, March CJ, Conlon PJ, Hopp TP, Gillis S, Uradal DL. Detection and characterization of high affinity plasma membrane receptors for human interleukin-1. J Exp Med 1985; **162**:501–15.
18 Lowenthal JW, Zubler RH, Nabholz M, MacDonald HR. Similarities between interleukin 2 receptor number and affinity on activated B and T lymphocytes. Nature 1985; **315**:669–72.
19 Schneider C, Newman RA, Sutherland R, Asser U, Greaves MF. A one step purification of membrane proteins using a high efficiency immunomatrix. J Biol Chem 1982; **257**:10766–9.
20 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; **227**:680–5.
21 Nagai S, Takeuchi M, Aung H, Kitaichi K, Izumi T. IL-1 inhibitory factor released from alveolar macrophages in interstitial lung diseases. Eur Respir J 1990; **3**:243s.
22 Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991; **77**:1627–52.
23 Solari R, Smithers N, Page K, Bolton E, Champion BR. Interleukin-1 responsiveness and receptor expression by murine TH1 and TH2 clones. Cytokine 1990; **2**:129–41.
24 Mancilla J, Ikejima I, Clark BD, Orencole SF, Sirko S, Dinarello CA. Lectin binding suggest different glycosylation patterns of IL-1 receptors on different cells. Cytokine 1990; **1**:95–108.
25 Carter DB, Deibel MR, Dunn CJ, *et al.* Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 1990; **344**:633–8.
26 Mazzei GJ, Seckinger PL, Dayer JM, Shaw AR. Purification and characterization of a 26-kDa competitive inhibitor of interleukin 1. Eur J Immunol 1990; **20**:683–9.
27 Rochemonteix BG, Nicod LP, Junod AF, Dayer JM. Characterization of a specific 20- to 25-kD interleukin-1 inhibitor from cultured human lung macrophages. Am J Resp Cell Mol Biol 1990; **3**:355–61.
28 Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990; **348**:550–2.
29 Ulich TR, Yin S, Guo S, Castillo J, Eisenberg SP, Thompson RC. The intratracheal administration of endotoxin and cytokines. III. The interleukin-1 (IL-1) receptor antagonist inhibits endotoxin- and IL-1-induced acute inflammation. Am J Path 1991; **138**:521–4.
30 Libby P, Warner SJ, Friedman GB, Interleukin-1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids. J Clin Invest 1988; **81**:487–98.