Production of IL-1 and its receptor antagonist is regulated differently by IFN-γ and IL-4 in human monocytes and alveolar macrophages

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ABSTRACT: Interleukin-4 (IL-4) has previously been found to downregulate interleukin-1 (IL-1) production, but to upregulate the production of IL-1 receptor antagonist (IL-1ra) in human monocytes stimulated with lipopolysaccharide (LPS). In the present study we wanted to determine whether the production of IL-1ra in human monocytes and alveolar macrophages (AMs) is regulated differently at the protein and messenger ribonucleic acid (mRNA) levels by IL-4 and interferon-γ (IFN-γ).

AMs and monocytes obtained from healthy donors by bronchoalveolar lavage and centrifugal elutriation were stimulated with LPS in the presence or absence of IL-4 or IFN-γ, and the expression of mRNA for IL-1 and IL-1ra was measured by Northern blot analysis. The production of IL-1 and IL-1ra was quantitated by enzyme immunoassays (EIAs).

Spontaneous IL-1ra production was seen in AMs after incubation for 4 h in medium alone, but not in blood monocytes, at both the protein and mRNA levels. The spontaneous expression of the IL-1ra gene in AMs was augmented by incubation with IL-4. Interleukin-1β (IL-1β) production by LPS-stimulated AMs and monocytes was upregulated by IFN-γ, but downregulated by IL-4. Interestingly, when stimulated with LPS, IFN-γ inhibited IL-1ra production by monocytes, but up-regulated its production in human AMs at the protein and mRNA levels.

These results suggest that the production of IL-1 and IL-1ra by monocyte-macrophages is regulated differently at the mRNA level, depending upon the balance between the production of IL-4 and IFN-γ at the sites of T-cell/macrophage interactions in the lung.

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Interleukin 1 (IL-1), a monokine produced primarily by macrophages, is known to be involved in the host response to injury and infection [1, 2]. Although inflammatory processes are defence mechanisms, IL-1 may cause tissue damage and may contribute to chronic inflammation in upregulated conditions. Therefore, its potent pleiotropic inflammatory effects are likely to be tightly regulated in vivo.

The in vivo action of IL-1 is controlled in several ways, e.g. by regulation of its synthesis and by production of neutralizing soluble factors, including soluble IL-1 receptor, anti-interleukin-1α (IL-1α) autoantibody and an antagonistic inhibitor at its receptor level [2–7]. The IL-1 receptor antagonist (IL-1ra), which specifically antagonizes the effect of IL-1 at its receptor level, was recently characterized and cloned [6, 7]. Since IL-1 and IL-1ra are produced by stimulated monocytes and tissue macrophages, it was important to examine the mechanism regulating IL-1ra production in comparison with that regulating IL-1 production.

T-cell derived cytokines interferon-γ (IFN-γ) and interleukin-4 (IL-4) have different regulatory effects on various functions of human monocytes and macrophages. For example, IFN-γ, as a macrophage-activating factor [8], primes human monocyte-macrophages to respond to secondary stimuli (e.g. lipopolysaccharide (LPS)) to express antitumour activity [9], and to produce monokines (IL-1 and tumour necrosis factor (TNF)) [10, 11]. On the other hand, IL-4, as a B-cell stimulatory factor [12], has been shown to suppress the abilities of LPS stimulated monocyte-macrophages to express tumour cytotoxicity [13], to secrete prostaglandin E2 (PGE2) [14], H2O2 [15] and O2 (16), and to produce IL-1, interleukin-6 (IL-6) and TNF-α [17–20]. In contrast, IL-4 was recently found to augment IL-1ra production by human blood monocytes and macrophages [21–23]. Thus, IL-4 may downregulate the IL-1-initiated host immune response not only by a direct inhibitory effect on monocytes, resulting in reduced production of IL-1, but also by upregulating the production of IL-1ra to antagonize the effect of IL-1.
The *in vivo* function of monocyte-macrophages probably depends on the balance between the productions of IL-4 and IFN-γ at the sites of T-cell/macrophage interactions. However, little is known about the regulatory effects of IFN-γ and IL-4 on IL-1ra production by human alveolar macrophages. We report that IL-1ra is a potent upregulator of human alveolar macrophages (AMs) and monocytes capable of producing IL-1ra, and that IFN-γ is an inhibitor of IL-1ra production by monocytes, but an upregulator of IL-1ra production by human AMs at the protein and messenger ribonucleic acid (mRNA) levels.

**Materials and methods**

**Subjects**

Cells were obtained from 12 healthy nonsmoking male donors, 20–25 yrs of age. These volunteers had no evidence by history or physical examination of lung disease, were not taking medication, gave normal results in pulmonary function tests and chest roentgenograms were normal. They all gave informed consent to participate in the experiment.

**Reagents**

RPMI 1640 medium (Nissui Chemical Co., Tokyo, Japan) supplemented with glutamine (1 mM) and gentamycin (50 µg·ml⁻¹) was used in all experiments. Recombinant human IL-4 (specific activity 1×10⁶ U·mg⁻¹ protein) was kindly provided by Ono Pharmaceutical Co., Osaka, Japan. Recombinant human IFN-γ (specific activity 5.36×10⁶ U·mg⁻¹) was a gift from Nippon Roche (Tokyo, Japan). None of the reagents contained endotoxins as judged by Limulus amoebocyte assay (Seikagaku Kogyo, Tokyo, Japan; minimum detection level 0.3 ng·ml⁻¹). Lipopolysaccharide (LPS; Seikagaku Kogyo, Tokyo, Japan) supplemented with glutamine (1 mM) and gentamycin (50 µg·ml⁻¹) sonicated salmon sperm DNA, 0.1% sodium dodecyl sulphate (SDS), and 5×Denhardt’s solution at 42°C was used to prepare the DNA probe. The yield of human AMs from normal volunteers was approximately 1.8×10⁷ viable cells per wedge segment (>93%) viable as determined by trypsin blue dye exclusion. Differential counts established that >89% of the lavaged cells were AMs (staining for nonspecific esterase). The other cells were either small mononuclear cells or neutrophils.

In vitro stimulation of monocytes and AMs

Monocytes (2×10⁶·ml⁻¹) and AMs (1×10⁶) were suspended in serum-free RPMI 1640 medium and incubated for 4 h at 37°C in suspension in plastic dishes (Falcon 3003; Becton Dickinson, Lincoln Park, NJ, USA) containing medium with or without LPS in the presence or absence of IL-4 or IFN-γ.

**Isolation of blood monocytes**

Mononuclear cells were harvested at the interface of an isotonic Ficoll cushion (specific gravity, 1.077) from a leucocyte-rich fraction obtained by leucapheresis. Then monocytes were isolated by counterflow centrifugal elutriation with a Beckman JE-5.0 system using the method described previously [11, 21]. The purity of the monocyte fraction was usually over 95%, as judged by morphological examination and nonspecific esterase staining, and the viability of the cells was over 98% as assessed by the trypan blue dye exclusion test.

**Harvesting and preparation of human AMs**

Bronchoalveolar lavage was performed as described in detail previously [11]. Briefly, after anaesthetizing the oral cavity and the upper airway with lidocaine spray, the tip of an Olympus fibreoptic bronchoscope (Model BF-1T20; Olympus Co., Tokyo, Japan) was wedged into one of the segments of the right or left lung. The lung was washed with 50 ml of sterilized saline (0.9% NaCl) prewarmed to 37°C, and the fluid was gently sucked out with a 50 ml syringe. This process was repeated three times. A total of 150 ml of saline was instilled, of which about 65% was recovered. The yield of human AMs from normal volunteers was approximately 1.8×10⁷ viable cells per wedge segment (>93%) viable as determined by trypsin blue dye exclusion. Differential counts established that >89% of the lavaged cells were AMs (staining for nonspecific esterase). The other cells were either small mononuclear cells or neutrophils.

**Northern blot hybridization analysis**

Total ribonucleic acid (RNA) was extracted from cells by the guanidium thiocyanate/caesium chloride method [24]. RNA blotting was carried out as described previously [21, 25]. Total RNA was denatured in a solution of 20 mM 3-(N-morpholino propanesulphonic acid (MOPS) buffer, pH 7.0, containing 6% formaldehyde, 50% formamide, 5 mM sodium acetate, and 1 mM ethylene diamine tetra-acetic acid (EDTA) at 65°C for 5 min, and then separated on 1.2% agarose gel containing 6% formaldehyde. It was then transferred to a Hybond-N nylon membrane (Amersham International Inc., UK) and hybridized with 32 P-labelled probes. The probes used were the PstI-Scal fragment (about 0.56 kb length) of complement deoxyribonucleic acid (cDNA) for the IL-1 receptor antagonist (IL-1ra), the PstI-PvuII fragment (0.67 kb) of cDNA for IL-1β, and the EcoRI-HincII fragment (0.45 kb) of cDNA for IL-1α. These probes were kindly provided by T. Nishida (Otsuka Pharmaceutical Co., Tokushima, Japan). The probes were labelled by the multiprime DNA labelling method [26]. Before hybridization, the nylon membrane was prehydrated in 50% formamide, 5×SSPE (SSPE; 0.15 M NaCl, 10 mM NaH₂PO₄, pH 7.4, 1 mM EDTA), 200 µg·ml⁻¹ sonicated salmon sperm DNA, 0.1% sodium dodecyl sulphate (SDS), and 5×Denhardt’s solution at 42°C for 6 h. Hybridization was performed at 42°C for 14 h in the same solution containing 10% dextran sulphate and labelled cDNA (10⁶ cpm·µg⁻¹) with a specific activity of about 10⁶ cpm·ml⁻¹. After hybridization, the membrane was washed four times with 2×SSPE containing 0.1% SDS for 15 min each time at 42°C, and then with 1×SSPE containing 0.1% SDS for 30 min at 42°C. The membrane was autoradiographed with Kodak XAR-5 film at -70°C with an intensifying screen.
These experiments were performed 4 h after the start of culture of monocytes or AMs with or without a stimulating agent. All experiments were repeated in at least three different donors to produce the similar results. In preliminary experiments, appreciable levels of mRNAs for IL-1β and IL-1ra were not detected in fresh AMs isolated by lavage from the lungs of four different normal nonsmokers (data not shown).

Quantitative analysis of autoradiograms

Autoradiograms obtained by Northern blot analyses were quantitated by densitometric scanning with a FUJIX Bio-Image Analyzer-BAS2000 (Fuji Photofilm Co., Japan).

Enzyme immunoassays (EIAs) of human IL-1α, IL-1β and IL-1ra

EIAs for human IL-1α, IL-1β and IL-1ra were performed essentially as described previously [11, 17].

Results

Difference in regulation by IL-4 of IL-1β and IL-1ra mRNA expression by AMs and monocytes

We examined whether IL-4 regulated the gene expression of IL-1β by AMs and monocytes in response to LPS in different ways. For this, AMs were incubated for 4 h in medium with or without IL-4, before Northern blot analysis. Data are shown in figure 1. When incubated for 4 h in serum-free medium alone, monocytes expressed little IL-1β mRNA, but AMs showed spontaneous
gene expression. Treatment for 4 h with an optimal concentration of LPS (0.1 µg·ml⁻¹) resulted in significant gene expression of IL-1β by monocytes and AMs. The level of IL-1β mRNA in AMs was less than that in monocytes. Under these conditions, the gene expression of IL-1β by LPS-stimulated monocytes and AMs was significantly suppressed by IL-4 (100 U·ml⁻¹).

We also examined the effect of IL-4 on the expression of IL-1ra gene by AMs and monocytes. IL-4 upregulated IL-1ra mRNA expression by LPS-stimulated monocytes (fig. 2). Under the same experimental conditions, AMs that had been incubated for 4 h in medium showed spontaneous gene expression of IL-1ra, and IL-4 alone upregulated the gene expression. LPS induced slight, but appreciable, increase in the level of IL-1ra mRNA. LPS and IL-4 caused marked increase in IL-1ra mRNA of monocytes, but no increase in IL-1ra mRNA of AMs over that with IL-4 alone (fig. 2).

Upregulation or downregulation by IFN-γ or IL-4 of IL-1ra mRNA expression by monocytes

We then examined whether IFN-γ and IL-4 affected the expression of mRNA for IL-1ra (fig. 3). IL-4 and IFN-γ did not change the expression of β-actin mRNA (data not shown). Addition of IL-4 to cultures of monocytes with LPS resulted in significant increase in IL-1ra mRNA expression. In contrast, IFN-γ reduced the expression of IL-1ra mRNA in LPS-stimulated monocytes.

Effect of IFN-γ on the expression of IL-1ra mRNA in AMs

Human AMs were incubated for 4 h in serum-free medium with or without LPS in the presence or absence of IFN-γ (100 U·ml⁻¹), before Northern blot analysis. As shown in figure 4, normal human AMs incubated in medium alone or with IFN-γ expressed appreciable levels of mRNA for IL-1α. AMs treated with either LPS or IFN-γ alone showed increased levels of IL-1α gene expression, and maximal expression on treatment with IFN-γ plus LPS. IL-1β mRNA expression was also augmented by treating AMs with LPS or IFN-γ plus LPS. Under the same experimental conditions, AMs expressed IL-1ra mRNA spontaneously. LPS or IFN-γ alone had little effect on the expression of IL-1ra mRNA by AMs, but LPS plus IFN-γ caused upregulation of IL-1ra mRNA expression in AMs.

Differential effects of IL-4 and IFN-γ on production of IL-1 and IL-1ra by monocytes and autologous AMs

For this, blood monocytes and AMs were incubated in triplicate cultures for 24 h in serum-free medium with
or without various agents, and then the cell-free supernatants were collected by brief centrifugation. Extracellular monokines in each sample of triplicate cultures were quantitated by the EIAs. The results are given in table 1. In serum-free medium with or without IL-4 or IFN-γ; neither monocytes nor AMs secreted appreciable amounts of IL-1 (α and β). The production of IL-1 by monocytes and AMs, activated with 0.1 µg·ml⁻¹ LPS was significantly suppressed by IL-4 (p<0.05), but augmented by IFN-γ (p<0.05). Under these experimental conditions, untreated monocytes did not secrete IL-1ra, but the autologous AMs spontaneously produced large amounts of IL-1ra in the culture supernatants. LPS enhanced IL-1ra production by monocytes and AMs. IL-1ra production by LPS-stimulated monocytes was augmented by IL-4, but reduced by IFN-γ. IL-1ra production by AMs, was significantly augmented by treatment with IL-4 or IFN-γ (p<0.05).

**Discussion**

This study showed that IL-4 inhibits IL-1 production by AMs as well as monocytes, but augments IL-1ra production at both protein and gene levels. Also, that although IFN-γ augments IL-1 production by monocytes and AMs in response to LPS, it inhibits IL-1ra production by LPS-stimulated monocytes, but augments its production by AMs at the protein and mRNA levels.

AMs are major effector cells in host defence of the lung, and play an important role in the regulation of inflammatory and/or immune responses [27, 28]. Cultured human lung macrophages were previously found to release an IL-1 inhibitor (20–25 kD protein) into their culture supernatant [29]. Recently, AMs were found to be a significant source of IL-1ra and to produce IL-1ra spontaneously in cultures without activation stimuli [32]. This observation was confirmed by the present finding of detectable levels of IL-1ra protein and its mRNA in AMs, but not in monocytes, that had been incubated for 4 h in medium alone, although freshly isolated monocytes and AMs of normal donors did not show detectable levels of IL-1ra mRNA. These findings indicate that production of IL-1ra by AMs may be important for the maintenance of homeostasis of alveolar spaces, by inhibiting their response in the lung to inflammatory stimuli, such as inhaled environmental agents. Moreover, LPS increased the expression of the IL-1α and IL-1β genes in human AMs, but did not increase IL-1ra expression fully.

The inability of human AMs to express the IL-1α gene fully in response to LPS may be due to the state of differentiation and/or maturation of monocyte-macrophages, because in vitro monocyte-derived macrophages were found to produce IL-1ra without activation stimuli, and to be nonresponsive to LPS or adherent immunoglobulin G (IgG) [30]. Another possible explanation is a difference in the activation and/or stimulation state, because human AMs, unlike monocytes, are already in a functionally stimulated and/or activated state in terms of tumour cytotoxicity and production of various monokines (IL-1, IL-6 and TNF-α) [11, 17, 19, 31, 32]. IFN-γ and IL-4 have different regulatory effects on cytokine production by human blood monocytes. IL-4 strongly inhibits the secretions of IL-1, IL-6, TNF and oxygen radicals by human monocytes and AMs [16–20]. Consistent with previous findings [21], IL-4 augmented IL-1ra production and its mRNA expression not only by monocytes, but also by autologous AMs (fig. 2 and table 1). In contrast, IFN-γ downregulated IL-1ra production by blood monocytes stimulated with LPS at both protein and gene levels (fig. 3 and table 1). This observation, together with previous findings of upregulating effects of IFN-γ on the productions of monokines, such as IL-1; TNF and IL-6 [8–10], suggest an important role for IFN-γ in augmentation of IL-1-initiated immune responses through direct and indirect cytokine networks. Nevertheless, it should be noted that IFN-γ

<table>
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<tr>
<th>Treatment of monocytes or AMs</th>
<th>Monocytes</th>
<th>AMs</th>
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<tbody>
<tr>
<td></td>
<td>IL-1α pg·ml⁻¹</td>
<td>IL-1β pg·ml⁻¹</td>
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<tr>
<td>Medium</td>
<td>&lt;10</td>
<td>&lt;20</td>
</tr>
<tr>
<td>IFN-γ 100 U·ml⁻¹</td>
<td>&lt;10</td>
<td>&lt;20</td>
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<tr>
<td>IL-4 100 U·ml⁻¹</td>
<td>&lt;10</td>
<td>&lt;20</td>
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<tr>
<td>LPS 0.1 µg·ml⁻¹</td>
<td>310*</td>
<td>793*</td>
</tr>
<tr>
<td>LPS + IL-4</td>
<td>112**</td>
<td>107**</td>
</tr>
<tr>
<td>LPS + INF-γ</td>
<td>496**</td>
<td>1066**</td>
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Values are the mean for triplicate cultures. The SDs were consistently <10% of the means. Data are representative of three separate experiments which produced the same results. *: p<0.05 vs value for monocytes or AMs incubated in medium alone; **: p<0.05 vs value for monocytes or AMs treated with LPS alone. AMs: alveolar macrophages; IL-1: interleukin-1; IL-1ra: interleukin-1 receptor antagonist; LPS: lipopolysaccharide; IFN-γ: interferon-γ; IL-4: interleukin-4.
did not inhibit, but rather upregulated IL-1ra production and its gene expression in normal human AMs. Our results reveal that in response to IFN-γ, AMs are important not only in producing IL-1, but also in producing large amounts of IL-1ra, thus indicating their critical role in both normal homeostasis in the lung and control of the pulmonary inflammatory response. Indeed, normal human AMs have been shown to suppress various immune responses in vitro, such as the mitogen response of T-lymphocytes and induction of IL-2-activated killer activity [33–35]. The physiological relevance of the difference between IFN-γ and IL-4 in regulation of IL-1 and its receptor antagonist (IL-1ra) in mature macrophages (AMs) is not yet known. The present results, together with the fact that AMs have limited ability to produce IL-1 [36], suggest that AMs may play an important role in regulating the local immune response of the lung. Thus, the magnitude of local production of either IFN-γ or IL-4 will dictate the eventual biological effect of IL-1 in the lung. Finally, the present findings indicate important immunoregulatory roles for IFN-γ and IL-4 in macrophage-driven pathogenesis of the lung.

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References


