Downloaded from www.sjweh.fi on March 22, 2017

*Original article*

Scand J Work Environ Health 1992;18(2):75-77

**Interleukin 1 and its inhibition in an inflammatory reaction caused by Aspergillus umbrosus.**

by Manninen A, Lewis DM, Olenchock SA, Sorenson WG, Mull JC, Whitmer M, Terho EO, Husman KR, Kotimaa M

**Affiliation:** National Institute for Occupational Safety and Health, Division of Respiratory Disease Studies, Morgantown, West Virginia.

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/1514094

Print ISSN: 0355-3140 Electronic ISSN: 1795-990X Copyright (c) Scandinavian Journal of Work, Environment & Health

*Scand J Work Environ Health* 1992;18 Suppl 2:75—7

# Interleukin 1 and its inhibition in an inflammatory reaction caused by *Aspergillus umbrosus*

by Alli Manninen, PhD,[1] Daniel M Lewis, PhD,[1] Stephen A Olenchock, PhD,[1] William G Sorenson, PhD,[1] Judith C Mull, MSc,[1] Michael Whitmer, BSc,[1] Erkki O Terho, MD,[2] Kaj RH Husman, MD,[3] Marjut Kotimaa, PhD[3]

Interleukin 1 (IL-1), a polypeptide, is a cytokine that is responsible for mediating processes in host defense, inflammation, and response to injury. It is produced by a variety of cells, activated macrophages, fibroblasts, keratinocytes of the skin, T and B lymphocytes, brain astrocytes, and microglial cells after infection or injury. IL-1 has activities similar to those of tumor necrosis factor alpha, lymphotoxin, and interleukin 6. Two biochemically distinct but structurally related IL-1 molecules have been cloned — IL-1 alpha and IL-1 beta.

Like other cytokines, IL-1 has various biological activities. It was first described as an endogenous pyrogen, produced by leukocytes, which induces fever. At present it is known that IL-1 affects the central nervous system, metabolism, blood cells, and vascular walls (1). IL-1 acts as an inducer of cytokines by stimulating the production of interleukin 2 (IL-2), the interferons, interleukin 3, other colony-stimulating factors in bone marrow, and B cell-stimulating factor 2. For immunologic responses, IL-1-induced production of itself, IL-2, and B lymphocyte growth factors augment the immune response to antigens (2).

IL-1 attracts leukocytes into inflamed tissues, causes degranulation of basophils and eosinophils, stimulates tromboxane synthesis in macrophages and neutrophils, and potentiates the activation of neutrophils by chemoattractant peptides. IL-1 is an immunostimulant because it directly activates lymphocytes and indirectly induces the synthesis of molecules which then activate lymphocytes (2).

Stimuli for IL-1 production include microorganisms, endotoxin (lipopolysaccharide), and antigen-antibody complexes. Lipopolysaccharide is one of the most potent stimuli for the production of IL-1 (3). *Aspergillus umbrosus* seems to be an etiologic factor for hypersensitivity pneumonitis, which is a disease resulting from prolonged exposure to a variety of organic material (4, 5). *Micropolyspora faeni,* another cause of hypersensitivity pneumonitis, was found to stimulate excessive IL-1 production in blood monocytes and alveolar macrophages from nonfarmer volunteers and in murine peritoneal macrophages (6).

Because IL-1 is highly inflammatory, the downregulation of its production could be beneficial for specific diseases like arthritis, endotoxemia, and hypersensitivity diseases. Therapeutics currently in use to treat the aforementioned diseases are corticosteroids. A new agent, pentamidine, has been demonstrated to inhibit the release of IL-1 from macrophages (7).

## *Materials and methods*

*A umbrosus* was provided for this study by the Kuopio Regional Institute of Occupational Health, Kuopio, Finland. It was cultured for three weeks at room temperature in culture medium based on malt extract. The suspension was filtered and washed three times by sterile, nonpyrogen water (Travenol Laboratories Inc, Illinois, United States). The mycelium was extracted into phosphate buffered saline, pH 7.4. The suspension was homogenized for 1 min three times, and the cells were disrupted by ultrasound for 45 s five times. Mycelium was separated from the suspension by centrifuging the suspension at 20 000 revolutions/min for 30 min at 4°C. Mycelium was discarded, and the supernate was concentrated by ultrafiltration (Diaflo, Amicon Corp, United States). Total protein was measured by the method of Lowry (8).

Male Sprague-Dawley rats (viral antibody-free) were used for this study. Each group included four rats. The animals were exposed to the antigen extract (100 $\mu l \cdot 100\ g^{-1}$ body weight) and antigen plus endotoxin (1 million EU $\cdot$ $ml^{-1}$). The controls were exposed to sterile, nonpyrogen 0.9% saline. The animals were lightly anesthetized by pentobarbital before the instillations. They were dosed three times three days apart.

*Bioassay of interleukin 1 from alveolar macrophages.* We obtained alveolar macrophages by lavaging the lungs with sterile, nonpyrogen 0.9% saline. The macro-

[1] National Institute for Occupational Safety and Health, Division of Respiratory Disease Studies, Morgantown, West Virginia, United States.

[2] University of Turku, Turku, Finland.

[3] Kuopio Regional Institute of Occupational Health, Kuopio, Finland.

Correspondence to: Dr A Manninen, Kuopio Regional Institute of Occupational Health, PO Box 93, SF-70701 Kuopio, Finland.

**Table 1.** Effect of intratracheal exposure to *Aspergillus umbrosus* on interleukin 1 (IL-1) activity in rat alveolar macrophages.

| Exposing agent | IL-1 activity (counts/min) | | Number of macrophages/ ml |
|---|---|---|---|
| | Mean | SD | |
| *A umbrosus* | 2005 | 318 | $2.6 \cdot 10^6$ |
| Saline control | 164 | 13 | $0.4 \cdot 10^6$ |

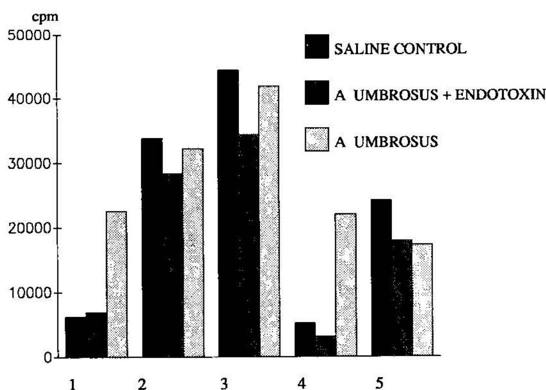


**Figure 1.** Interleukin 1 (IL-1) activity in rat alveolar macrophages after intratracheal exposure of rats to *Aspergillus umbrosus* and endotoxin followed by the additional stimulation of macrophages by endotoxin (1 and 5 µg · $ml^{-1}$) and inhibition by pentamidine (10 µM). (1 = culture medium, 2 = 1 µg endotoxin · $ml^{-1}$, 3 = 5 µg endotoxin · $ml^{-1}$, 4 = 10 µM pentamidine, 5 = 1 µg endotoxin · $ml^{-1}$ plus 10 µM pentamidine, cpm = counts per minute)

phages from four animals were pooled into one group to get enough cells for the assay. The macrophages were allowed to adhere to plastic culture wells (tissue culture clusters, Costar) in serum-free RPMI 1640 (RPMI = Rothwell Park Memorial Institute) containing 2 mM L-glutamine and 10 000 units of pennstrep and mycostatin for 1 h. Nonadherent cells were removed by rinsing with culture medium. The IL-1 assay was conducted on one group of exposed animals and one group of unexposed animals. Macrophages from the animal groups exposed in vivo to *A umbrosus* and *A umbrosus* plus endotoxin were used for the in vitro assays. Cells were exposed to culture medium, endotoxin (1.0 and 5.0 µg · $ml^{-1}$), pentamidine (10 µM), and pentamidine (10 µM) followed by endotoxin (1.0 µg · $ml^{-1}$) for 16 h at 37°C in 5% carbon dioxide. The plates were frozen at −85°C. The IL-1 activity was determined by functional assay using murine thymocytes (CD-1 mice, 6—8 weeks of age). Thymocytes in culture medium (100 ml) containing 0.2 mM 2-mercaptoethanol, was added to the wells, one million cells/well. Every plate had a positive control with a known amount of IL-1. The plates were incubated for 42 h at 37°C in 5% carbon dioxide. The cells were incubated with $^3$H-thymidine (1 µCi · $well^{-1}$, New England Nuclear) and harvested onto glass fiber filters by a PHD cell harvester (Cambridge Technology Inc, Maine, United States). Radioactivity was counted with a liquid scintillation counter (1214 Rackbeta, Wallac, Turku, Finland).

## *Results and discussion*

Initial studies were done to determine whether *A umbrosus* could stimulate IL-1 release from rat alveolar macrophages stimulated in vivo followed by in vitro exposure. The results showed that exposure to *A umbrosus* increased IL-1 activity 12-fold in alveolar macrophages in vivo (table 1). The total cell counts showed that the exposure caused an inflammatory condition in the lungs because the number of macrophages in the lavage fluids of the exposed animals increased from 0.4 million · $ml^{-1}$ to 2.6 million · $ml^{-1}$. In previous experiments 70% of the cells were lymphocytes and 20—30% were macrophages in the exposed animals (data not shown).

Exposures to *A umbrosus* and endotoxin followed by in vitro stimulation showed that *A umbrosus* could increase IL-1 activity more than exposure to *A umbrosus* plus endotoxin. Because lipopolysaccharide is considered to be a very potent IL-1 stimulant, endotoxin was used as an additional stimulant in order to test the inhibitory effect of pentamidine for IL-1. Additional stimuli by endotoxin increased IL-1 activity in the macrophages of all the groups (figure 1). The higher dose of endotoxin did not significantly enhance the IL-1 activity. Pentamidine effectively decreased IL-1 in the groups of combined exposure to endotoxin and *A umbrosus,* but it did not inhibit IL-1 as well in only antigen-exposed animals. Corticosteroids, which are generally used for treating granulomatous lung diseases, inhibited IL-1 at the transcriptional level, but pentamidine, an aromatic diamine, inhibited the release of cellular IL-1 as previously shown (7).

In conclusion, *A umbrosus* can cause an IL-1-mediated inflammatory reaction like endotoxin or *M faeni. M faeni,* like *A umbrosus,* is a causative agent of hypersensitivity pneumonitis, an immunologically induced inflammation of the lung interstitium, terminal bronchioles and alveoli. This result provides some evidence that IL-1 may also be involved in the pathogenesis of hypersensitivity pneumonitis.

## *Acknowledgments*

This study received financial support from the Academy of Finland, the Finnish Work Environment Fund, the Allergy Research Foundation, and the Association of Promotion of Occupational Health.

## *References*

1. Dinarello CA. Biology of interleukin 1. FASEB J 1988; 2:108—15.
2. Dinarello CA. Interleukin-1 and its biologically related cytokines. Adv Immunol 1989;44:153—205.
3. Morin M, Schindler R, Wakabayashi G, Daumy G,

Dinarello CA, Gelfand JA. Picogram concentrations of endotoxin stimulate synthesis of IL-1 beta and TNF alpha by human peripheral blood mononuclear cells exposed to recombinant human C5a. Eur Cytokine Network 1991; 2:27—30.
4. Terho EO, Husman K, Vohlonen I, Mäntyjärvi R. Serum precipitins against microbes in mouldy hay with respect to age, sex, atopy, and smoking of farmers: work-related respiratory diseases among Finnish farmers. Eur J Resp Dis Suppl 1987;152:115—21.
5. Manninen A, Vallyathan V, Olenchock SA, Lewis DM, Sorenson WG. Hypersensitivity pneumonitis (HP) in rats caused by *Aspergillus umbrosus* and *Thermoactinomyces vulgaris*. FASEB J 1991;5:A1010.
6. Denis M, Cormier Y, Tardif J, Ghadirian E, Laviolette M. Hypersensitivity pneumonitis: whole *Micropolyspora faeni* or antigens thereof stimulate the release of proinflammatory cytokines from macrophages. Am J Respir Cell Mol Biol 1991;5:198—203.
7. Rosenthal GJ, Corsini E, Craig WA, Comment CE, Luster MI. Pentamidine: an inhibitor of interleukin-1 that acts via a post-translational event. Toxicol Appl Pharmacol 1991;107:555—61.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265—75.