

Measurement of interleukin 1 in pulmonary reactions induced by agricultural dusts¹

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Occupational exposure to organic dusts occurs in a variety of work sites and is frequently associated with respiratory reactions. The types of reactions range from acute febrile illnesses such as grain fever, mill fever, and organic dust toxicity syndrome to chronic diseases such as byssinosis and hypersensitivity pneumonitis. The relationship between these acute illnesses and the chronic diseases is unclear, but both exposure dose and duration are important. The acute illnesses are seen the most frequently following a brief but intense exposure to organic dusts contaminated with microorganisms (bacteria or fungi). The chronic illnesses are associated with repeated exposures to organic dusts. In both organic dust toxicity syndrome and hypersensitivity pneumonitis the symptoms reported include fever, chills, myalgia, headache, chest tightness, dyspnea, and eye and throat irritation (1).

Interleukin 1 (IL-1) is a monokine produced by several cell types, but principally by monocytes and macrophages. It has been well documented that many of the signs and symptoms associated with inflammation (fever, chills, myalgia, and malaise) are mediated by IL-1 and that IL-1 has a critical role in the initiation of an immune response (2). For these reasons we decided to investigate whether extracts of organic dusts associated with both acute and chronic respiratory diseases can induce IL-1 production. Those substances that are potent at inducing IL-1 production might be more likely to cause a reaction of organic dust toxicity syndrome or, with chronic low-level exposure, induce an immune response that leads to a chronic disease such as hypersensitivity pneumonitis.

Previous studies from our laboratory have demonstrated that rat alveolar macrophages can be stimulated to produce IL-1 and that the quantity of IL-1 produced by the macrophages is dependent on the type of extract used to stimulate the macrophages (3). Macrophage IL-1 production in rats was monitored with a

bioassay that involved the proliferation of mouse thymocytes. In this assay system we found that extracts of environmental agents could have a direct effect on the mouse thymocytes, and therefore interassay comparisons were difficult. We attempted to circumvent these problems by using human peripheral blood monocytes and an immunochemical assay to quantify IL-1 production directly (4). The human monocyte assay system was limited in that the IL-1 response varied greatly between the individuals who donated the monocytes. Therefore we sought to develop a more consistent assay utilizing a histiocytic lymphoma cell line, U937, that could be maintained in continuous culture (5).

The comparison of the IL-1 response of U937 cells and peripheral blood monocytes is the subject of this report. In addition, the assessment of IL-1 levels in serum samples was evaluated to determine if this assay could be utilized in support of field investigations of pulmonary diseases occurring in the workplace.

Materials and methods

We accomplished the analysis of IL-1 beta (IL-1 β) in blood by spiking blood samples, plasma, or serum with a known quantity of IL-1 β , allowing the blood or plasma to clot, and then analyzing the IL-1 content of the samples. The experiment was designed so that the effect of (i) proteolytic enzymes activated during clotting, which might alter IL-1 β levels, or (ii) autocrine effects of IL-1, which might elevate IL-1 β levels, could be evaluated. The blood samples were divided into three portions. One sample was allowed to clot by the addition of calcium, and the serum was assayed for IL-1 content before and after the addition of a known quantity of IL-1. The second sample was centrifuged, and the plasma was collected and analyzed for IL-1 before and after spiking. The third whole blood sample was spiked with IL-1, and both the serum and plasma derived from it were analyzed. Blood samples were collected from five healthy volunteers, and the resulting serum or plasma samples were analyzed as follows.

The induction of IL-1 β production was evaluated with the use of human peripheral blood mononuclear cells (PBMC) harvested from blood by buoyant density centrifugation using 1.078 g · ml⁻¹ medium of either percoll or ficoll-hypaque. The PBMC were washed twice in Hank's balanced salt solution (HBSS)

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and resuspended to a concentration of $5 \cdot 10^6$ per milliliter in RPMI-1640 (RPMI = Roswell Park Memorial Institute) culture medium (Whittacre-MA Bioproducts, Walkersville, Maryland, United States) containing 10% fetal calf serum, streptomycin ($100 \mu\text{g} \cdot \text{ml}^{-1}$), and penicillin ($100 \text{U} \cdot \text{ml}^{-1}$). One hundred microliters of the PBMC suspension was added to each well of a 96-well culture plate and incubated for 2 h at 37°C in 5% carbon dioxide. Nonadherent cells were removed by washing the wells three times with fresh medium. The adherent cells (primarily monocytes) were incubated overnight in the aforementioned manner in 200 μl of the same medium containing a dilution of test extract or endotoxin sample. The plates were taken through one freeze-thaw cycle to release intracellular IL-1 β , and the quantity of IL-1 β in the medium was assayed as follows. The experiments using U937 cells were carried out in exactly the same manner except that the U937 cells were resuspended to a cell concentration of $5 \cdot 10^5$ per milliliter, and 100 μl of the cell suspension was added to each well, along with 100 μl of the diluted extract or endotoxin. IL-1 β was measured using a commercial enzyme-linked immunosorbent assay according to manufacturer recommendations (Cis-tron Biotechnology, Pine Brook, New Jersey, United States). The assay is reproducible and can detect 20 pg IL-1 $\beta \cdot \text{ml}^{-1}$. In each assay, the culture medium was used as the blank or negative control, and medium from cells cultured without any stimulant served as a control for spontaneous IL-1 β release. The spontaneous release value was subtracted from the values of all the samples so that the results reflected the quantity of IL-1 β produced in response to the stimulant added.

Results and discussion

The analysis of the IL-1 β content of blood and plasma revealed that the assay was not reliable for this purpose. One of five plasma samples and two of five serum samples contained detectable levels endogenously even though the subjects were in apparent good health. In one case the serum had detectable levels even though the plasma from which it was derived did not. Samples of whole blood or plasma that were spiked with IL-1 and then allowed to clot contained 65 to 90% more IL-1 than predicted. Only samples of plasma or serum that were analyzed directly after the addition of the IL-1 yielded results that were consistent with the levels predicted. These results suggest that proteolysis occurring during the clotting process artificially elevated the IL-1 levels, possibly by revealing more immunoreactive domains on the IL-1 β molecule. These results coupled with the relatively short half-time of the molecule in circulation indicated that the analysis of IL-1 in serum or plasma is not sufficiently reliable to be of use as a biomarker of pulmonary inflammation in humans at the present time.

The utility of using U937 cells to test IL-1-inducing activity was evaluated in a series of experiments. Pu-

rified lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 was used to stimulate IL-1 production by both peripheral blood monocytes and U937 cells. The U937 cells consistently produced more IL-1 than the monocytes (maximal IL-1 concentrations of $\sim 2000 \text{pg} \cdot \text{ml}^{-1}$ for the U937 cells compared with $\sim 1000 \text{pg} \cdot \text{ml}^{-1}$ for the monocytes). The concentration of LPS required to stimulate the half maximal response (EC_{50}) was calculated for two experiments. The EC_{50} was 7.5 and 12.0 ng LPS $\cdot \text{ml}^{-1}$ for the U937 cells and 1.0 and 4.6 ng LPS $\cdot \text{ml}^{-1}$ for the monocyte. This result indicates that the monocytes might be slightly more sensitive to LPS. The overall shape of the dose-response curves was similar for both cell types, the responses reaching a plateau around $100 \text{ng} \cdot \text{ml}^{-1}$ LPS. These results showed that the U937 cells gave a dose-dependent response to LPS and that they may be useful in the evaluation of environmental samples.

To evaluate the response of U937 cells to environmental samples, seven extracts of bulk cotton samples that had been previously tested using monocytes were tested with the U937 cells. The results of that study are shown in table 1. The response of the U937 cells was much more variable than that with the monocytes. With both cell types there was a trend for greater IL-1 production with samples that had higher endotoxin levels; however in neither case was the correlation coefficient significant ($r=0.777$ for monocytes and $r=0.705$ for U937 cells). The stimulation of the two cell types was done on separate occasions, and the extracts were stored at -85°C between experiments. The freezing of these samples may have altered their reactivity, and this experiment will be repeated so that both cell types are tested at the same time. The results do demonstrate that the U937 cells respond to extracts of environmental samples by producing IL-1. Additional studies are necessary to determine if the IL-1 response by these cells correlates with the inflammatory potential of the extracts.

In summary, the results of this study demonstrate that, while IL-1 can be measured in serum and plasma, the values may be artificially elevated. This finding implies that circulating levels of IL-1 may not be a biomarker of pulmonary inflammation that can be used in field investigations. It may be useful in exposure studies where the change in IL-1 levels, rather than the absolute value, may be meaningful. In addi-

Table 1. Interleukin 1 (IL-1) response to cotton extracts — comparison with monocytes and U937 cells.

Sample	Endotoxin (EU $\cdot \text{ml}^{-1}$)	IL-1 produced	
		Monocytes (pg $\cdot \text{ml}^{-1}$)	U937 (pg $\cdot \text{ml}^{-1}$)
Bulk cotton XJ-1	36	358	11
Bulk cotton SD-1	34	298	443
Carded yarn	167	834	1511
Bulk cotton, Sudan	3	118	0
Bulk cotton XJ-2	4.2	622	12.1
Bulk cotton E-2	3.4	289	0
Exhaust dust	88	663	2962

tion, we have found that the histiocytic cell line U937 will produce IL-1 in response to environmental samples and that it may be useful in the evaluation of the inflammatory potential of such samples.

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