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Interleukin 1 Bioactivity in the Lungs of Rats with Monocrotaline-Induced Pulmonary Hypertension[1] (42632)

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Abstract. A single subcutaneous injection of monocrotaline in rats provokes lung injury, inflammation, and progressive pulmonary hypertension. The specific mediators of the lung injury and inflammation and the relation of these events to the ensuing hypertensive pulmonary vascular disease are not understood. Since the monokine interleukin 1 (IL-1) has been implicated in acute inflammatory reactions, the present study tested the hypotheses that monocrotaline promotes the appearance of IL-1 in the bronchoalveolar spaces of treated rats and that accumulation of the monokine coincides temporally with development of lung injury, inflammation, and/or pulmonary hypertension. As expected, monocrotaline administration was associated with an early phase of pulmonary edema, manifest at Day 7 post-treatment as an increase in the lung wet-to-dry weight ratio, followed at Day 14 post-treatment by development of pulmonary hypertension as evidenced by progressive right ventricular hypertrophy. Lung inflammation also was present at Days 14 and 21 after monocrotaline as indicated by the accumulation of leukocytes in the bronchoalveolar lavage fluid and by an increase in the lung tissue activity of the granulocyte-specific enzyme myeloperoxidase. Interleukin 1, bioassayed in bronchoalveolar lavage fluid using the standard D10 T-cell assay system, was increased slightly at Day 4 postmonocrotaline, returned to baseline at Day 7, and was markedly elevated at Days 14 and 21 after monocrotaline treatment. These observations indicate that increases in the bronchoalveolar lavage fluid content of IL-1 bioactivity are temporally related to the evolution of monocrotaline-induced lung injury, inflammation, and pulmonary hypertension and suggest that the monokine may play a pathogenetic role in these events.

Monocrotaline, a pyrrolizidine alkaloid, causes lung injury and pulmonary edema (1), inflammation (2), and progressive pulmonary hypertension (3) in rats. Time course studies indicate that the lung injury and edema occur relatively early after monocrotaline administration, within approximately 1 week, while the progressive hypertensive pulmonary vascular remodeling and pulmonary hypertension become apparent at 1–3 weeks post-treatment. Recently, Stenmark and associates (2) have shown that an influx of inflammatory cells into the airways and attendant elaboration of leukotriene C_4 coincides in time with development of pulmonary hypertension. The specific chemical mediators of lung injury, pulmonary edema, and inflammation in the monocrotaline model as well as the relation of these events to the development of pulmonary hypertension are not understood. Because the pathology of monocrotaline-induced pulmonary hypertension in rats mimics to some degree primary pulmonary hypertension in humans, studies in the animal model may provide insight into mechanisms of the human disorder.

The monokine interleukin (IL-1) exhibits a number of biologic effects that implicate it as a mediator of monocrotaline-induced pulmonary hypertension. For example, IL-1 is chemotactic for granulocytes and, via a primary action on microvascular endothelium, promotes increased granulocyte–endothelial adherance (4). We recently have demonstrated in rabbits that a murine monocyte-derived IL-1 preparation causes avid pulmo-

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nary leukostasis (5) and, under certain conditions, pulmonary edema ((6) and submitted for publication). IL-1 also provokes fibroblast proliferation (7). This observation is of potential significance because pulmonary interstitial fibroblasts may elaborate collagen or differentiate into new pulmonary arterial smooth muscle cells, either of which could underlie some of the adverse structural alterations characteristic of hypertensive pulmonary vascular disease. In an initial effort to determine if the monokine participates in monocrotaline-induced pneumotoxicity, this study evaluated bronchoalveolar lavage fluid from treated rats for the presence of bioassayable IL-1 and sought temporal relationships between accumulation of IL-1 activity in lavage fluid and monocrotaline-induced pulmonary edema, inflammation, and pulmonary hypertension.

Materials and Methods. *Monocrotaline treatment and experimental protocol.* Adult male Sprague–Dawley rats weighing 225–250 g were randomly segregated into groups of four to six animals. Four groups were given a single subcutaneous injection of 105 mg monocrotaline per kilogram body weight. A fifth group was given an injection of the vehicle for monocrotaline. Monocrotaline and its vehicle were prepared as described previously (9). The four groups of treated animals were studied at 4, 7, 14 or 21 days postmonocrotaline. One or two vehicle control rats were studied at each of these time points as well.

On the day of study, rats were killed with an overdose of intraperitoneal sodium pentobarbital (100 mg/kg). Subsequently, the chest was opened and the lungs and heart were removed *en bloc.* A ligature was placed around the right mainstem bronchus and right pulmonary artery and the right lung removed for determination of the wet-to-dry weight ratio and for assessment of lung myeloperoxidase activity as described below. The left lung was then lavaged with five 3-ml aliquots of sterile saline. A total of 15 ml was administered and 11–12 ml was recovered. There were no differences between experimental groups in terms of the amount of lavage fluid recovered. As described below, the bronchoalveolar lavage fluid (BALF) was evaluated for the presence of IL-1 bioactivity and for the presence of inflammatory cells. After the lavage procedure, the heart was dissected free and reserved for determination of the extent of right ventricular hypertrophy. We then evaluated temporal relationships between the appearance of IL-1 bioactivity in BALF with several well-defined events associated with monocrotaline-induced pulmonary hypertension, including the development of an early phase of pulmonary edema, pulmonary inflammation, and progressive right ventricular hypertrophy.

Evaluation of pneumotoxicity and interleukin 1-like bioactivity. A gravimetric technique was used to assess the extent of pulmonary edema in control and monocrotaline-treated rats (10). In brief, the lower lobe of the right lung was weighed wet and then allowed to stand at room temperature until a stable dry weight was obtained. The ratio of the blood-inclusive wet lung weight to dry lung weight was used as an index of lung water accumulation.

The extent of right ventricular hypertrophy was assessed in the following manner. After the atria were trimmed away from the heart, the right ventricular free wall (RV) was carefully separated from the left ventricle plus septum (LV + S). The ratio RV/LV + S was then used as an index of the extent of right ventricular hypertrophy.

The extent of pulmonary inflammation in control and monocrotaline treated rats was evaluated in two ways. First, the total number of leukocytes were determined in BALF. In these instances, pooled BALF was centrifuged and the resultant pellet was resuspended in 1.0 ml Hanks' balanced salt solution. The total leukocyte count in an aliquot of resuspended BALF was determined using a Coulter particle counter. As a means of quantitating the extent of pulmonary leukostasis, the content of the granulocyte-specific enzyme myeloperoxidase was determined in lung tissue using a technique devised and validated by Goldblum *et al.* (10). In brief, the two upper lobes of the right lung were homogenized on ice in 0.5% hexadecyltrimethylammonium bromide (HTAB) in 50 m*M* sodium phosphate buffer (pH 6.0, 5.0 ml HTAB/gram lung tissue). After centrifugation at 40,000*g* for 10 min at 4°C, the supernatant was discarded and the pellet was

resuspended in HTAB. The resuspension was then frozen–thawed, homogenized, sonicated, and centrifuged as described above for four additional freeze–thaw cycles. Myeloperoxidase activity was determined spectrophotometrically.

The content of IL-1 bioactivity was determined in BALF using the IL-1-dependent proliferation of the T-cell clone D10.G4.1 reported by Kaye and co-workers (11). In brief, 0.1-ml aliquots of BALF were added in triplicate to 96-well plates which were subsequently sterilized by exposure to 5000 rad from a Cs irradiator. D10 T cells, 2×10^4 cells/well, were then added in a volume of 100 μl in the presence of 20 μg/ml of anti-idiotypic T-cell-receptor antibody (3D3). Plates were cultured for 48 hr and then pulsed with 2 μCi of [*methyl*-^{3}H]thymidine during the last 4 hr of culture. Cells were harvested and proliferation was determined by the extent of [^{3}H]thymidine incorporation. Activity is expressed as the counts per minute (cpm) observed in D10 cells treated with BALF minus the background activity observed in control D10 not exposed to BALF samples.

Statistics. Data are expressed as the means ± the standard error. Time- and experimental group-dependent differences in mean values were evaluated by two-way analyses of variance combined with Dunnett's test for multiple comparisons when appropriate. A P value equal to or less than 0.05 was considered to denote statistical significance.

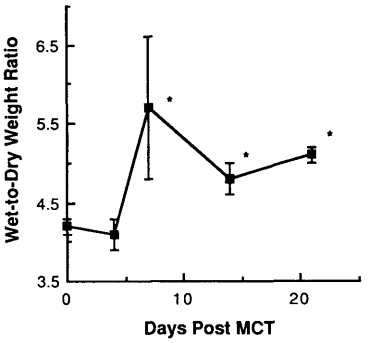

FIG. 1. Time course of changes in the lung wet-to-dry weight ratio in control and monocrotaline-treated (MCT) rats. Since values from control animals did not vary as a function of time, these values were pooled and designated as time = 0. N = 4–6 animals at each time point. *Significantly different from T = 0 (control) at $P < 0.05$.

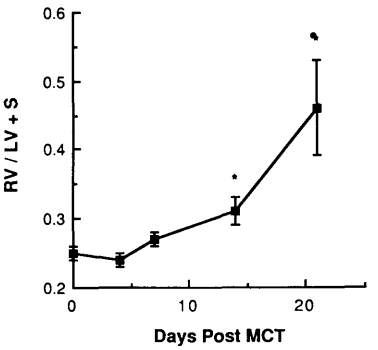

FIG. 2. Time course of development of right ventricular hypertrophy, expressed as the ratio of the right ventricular free wall weight (RV) to that of the left ventricle plus septum (LV + S), in monocrotaline (MCT)-treated and control rats. Since values from control animals did not vary as a function of time, these values were pooled and designated as time = 0. N = 4–6 animals at each time point. *Significantly different from T = 0 (control) at $P < 0.05$.

Results. *Monocrotaline-induced pneumotoxicity.* Time courses of changes in the lung wet-to-dry weight ratio and the evolution of right ventricular hypertrophy in monocrotaline-treated rats are shown in Figs. 1 and 2, respectively. At 7 days postmonocrotaline, the lung wet-to-dry weight ratio was significantly elevated above control and remained so throughout the 21-day duration of the study. The development of right ventricular hypertrophy, as evidenced by increases in the RV/LV + S ratio above control values, was apparent by Day 14 postmonocrotaline treatment. The ratio continued to increase throughout Day 21 after monocrotaline administration.

The development of pulmonary inflammation in monocrotaline-treated rats is depicted in Figs. 3 and 4. Relative to control animals, the content of leukocytes in BALF (Fig. 3) was increased at 14 days postmonocrotaline treatment. By Day 21, however, the BALF leukocyte burden was slightly lower in treated animals than in controls. Lung myeloperoxidase activity, an index of azurophilic granule-containing granulocytes, was increased at Day 21 postmonocrotaline relative to control animals (Fig. 4).

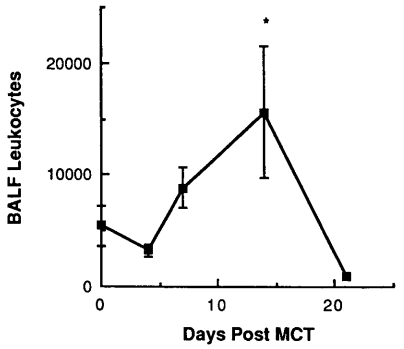

FIG. 3. Time course of changes in total bronchoalveolar lavage (BALF) leukocytes in monocrotaline (MCT)-treated and control rats. Since control values did not change as a function of time, these values were pooled and designated as time = 0. N = 4–6 animals at each time point. *Significantly different from T = 0 (control) at $P < 0.05$.

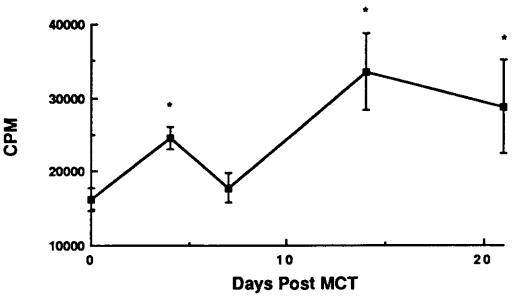

FIG. 5. Time course of changes in bronchoalveolar lavage fluid content of interleukin 1 activity, expressed as uptake of radioactive thymidine in cultures of D10 T cells, in monocrotaline (MCT)-treated rats. Since values from control animals did not change as a function of time, these values were pooled and designated as time = 0 (control). N = 4–6 animals per time point. *Significantly different from T = 0 (control) at $P < 0.05$.

Bronchoalveolar lavage fluid content of interleukin 1-like bioactivity. Time-dependent changes in the BALF content of IL-1-like bioactivity are shown in Fig. 5. Interleukin 1-like activity was increased at Day 4 post-monocrotaline but had returned to control levels by Day 7. At Day 14 post-treatment, however, IL-1-like activity in BALF was increased again and remained so throughout the 21-day duration of the study. Since a serum inhibitor of the D10 assay may exist (12), additional experiments were conducted to determine whether this inhibitor was present in BALF from monocrotaline-treated rats. A standard amount of murine monocyte-derived IL-1 was added to serial dilutions of BALF from treated animals and the activity of these mixtures was assessed in the D10 assay system. No inhibitory activity was detected at any dilution of monocrotaline-treated rat BALF (data not shown). It is thus unlikely that BALF from monocrotaline-treated rats contains an inhibitor of the D10 assay.

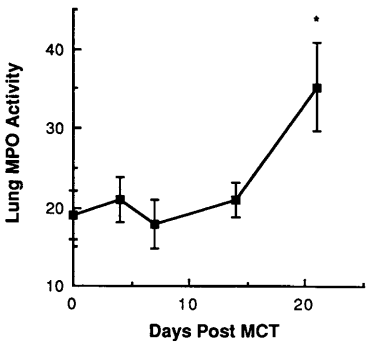

FIG. 4. Time course of changes in lung granulocyte burden, evaluated as lung myeloperoxidase activity (change in absorbance/minute/gram lung tissue) in monocrotaline (MCT)-treated rats. Since values from control animals did not change as a function of time, these values were pooled and designated as time = 0 (control). N = 4–6 animals at each time point. *Significantly different from T = 0 (control) at $P < 0.05$.

Discussion. In the present study, a single subcutaneous injection of monocrotaline in rats provoked an early increase in the lung wet-to-dry weight ratio indicative of pulmonary edema, pulmonary inflammation, as evidenced by the appearance on inflammatory cells in BALF and by an increase in the lung tissue burden of the granulocyte-specific enzyme myeloperoxidase, and progressive pulmonary hypertension manifest as progressive right ventricular hypertrophy. The time courses of these alterations are compatible with those reported by other investigators. For example, Sugita and co-workers (1) demonstrated that within 3 to 7 days after monocrotaline administration, there is an increase in the lung wet-to-dry weight ratio accompanied by increased extravasation of albumin into lung interstitium. The time course and importance of pulmonary inflammation in monocrotaline-treated rats

has been evaluated by Stenmark *et al.* (2). An increased BALF content of leukocytes, including granulocytes, abnormal alveolar macrophages, and inflammatory mediators such as peptidoleukotrienes, was associated and, in some instances, occurred in parallel with increases in pulmonary arterial pressure and development of right ventricular hypertrophy. Finally, numerous investigators have documented the existence of right ventricular hypertrophy in this model of lung injury and pulmonary hypertension and confirmed that the hypertrophy is temporally related to the evolution of hypertensive pulmonary vascular disease (e.g., (3)).

The principle observations of this study are that increased amounts of IL-1 bioactivity can be detected in BALF from monocrotaline-treated rats and that this increase appears to be temporally associated with well-defined pathologic events. In this context, increased IL-1-like bioactivity was first evident at 4 days postmonocrotaline and preceded slightly the increase in the lung wet-to-dry weight ratio indicative of pulmonary edema formation. After returning to control levels at Day 7, the BALF content of IL-1-like activity increased progressively from Days 14 through 21 postmonocrotaline. This latter time period coincides with development of a pulmonary inflammatory response and the evolution of hypertensive pulmonary vascular disease with progressive right ventricular hypertrophy.

There are several potential limitations of this study which engender caution in data interpretation. The first relates to the fact that proliferation of D10 T cells is influenced by interleukin 2 (IL-2) as well as IL-1. Since it is not known whether IL-2 was elaborated into the airways of monocrotaline-treated rats, the potential obfuscating influence of this lymphokine must be emphasized. It is noteworthy, however, that preliminary experiments conducted in our laboratories failed to detect IL-2, at least in BALF from rats with well-developed monocrotaline-induced pulmonary hypertension (unpublished observations). An additional limitation of the present study is that the results do not address the key issue of whether the increase in IL-1-like activity in BALF reflects increases occurring in the vicinity of target lung cells. In this regard, evaluation of bronchoalveolar lavage has been shown to provide information on the magnitude and type of cellular responses in other types of lung disease (13), although it is reasonable to suspect that in many instances changes in BALF contents of a given autacoid may lag behind changes in the interstitial content. Finally, it is appropriate to emphasize that the present results, although suggesting that a temporal association may exist between accumulation of an IL-1-like substance in BALF and certain aspects of monocrotaline-induced pneumotoxicity, do not address the relevant issue of whether the monokine plays a causal role in any of these events. However, for the reasons described below it is tempting to speculate that the monokine does indeed participate in the pathogenesis of monocrotaline-induced pulmonary hypertension.

If IL-1 is a mediator of monocrotaline-induced pneumotoxicity, then the monokine should exhibit biologic activities that are consistent with the major pathologic events characteristic of this model of lung injury and pulmonary hypertension. In this regard, IL-1 acts on endothelial cells to enhance adhesiveness for granulocytes, to promote elaboration of platelet-activating factor and plasminogen-activating factor inhibitor (and perhaps other injurious substances), and to cause expression of procoagulant activity (14–16). Additionally, IL-1 is chemotactic for granulocytes and may induce granulocyte oxidative metabolism (17, 18). It is thus reasonable to suspect that IL-1, through its action on endothelium and inflammatory cells, could play a key role in the development of monocrotaline-induced pulmonary edema and inflammation. In support of this contention, we recently have shown in rabbits that a murine monocyte preparation containing IL-1 activity provokes avid pulmonary leukostasis (5) and, under some conditions, pulmonary edema ((6) and submitted for publication). Indirect evidence also implicates IL-1 in monocrotaline-induced hypertensive pulmonary vascular disease. For example, in another model of pulmonary hypertension in rats which induced by exposure to chronic hypoxia, fibroblasts are suspected progenitors of the new arterial smooth muscle which underlies, in part, the

development of medial arterial thickening characteristic of hypertensive pulmonary vascular disease (19). Since medial arterial thickening also occurs in the lungs of monocrotaline-treated rats (3), and further, since hyperplasia of resident or itinerant lung fibroblasts may participate in this response as well, the mitogenic actions of IL-1 on fibroblasts (7) could play a key role in monocrotaline-induced hypertensive pulmonary vascular remodeling. Similarly, the proliferative effects of IL-1 on fibroblasts could be involved with the augmented perivascular collagen deposition recently reported in monocrotaline-treated rat lungs (20).

The present study did not attempt to identify the source of the increased BALF content of IL-1-like bioactivity. However, if IL-1 plays a role in monocrotaline-induced pneumotoxicity, it should be elaborated by a resident lung cell or an itinerant cell recruited to the lung in response to monocrotaline challenge. In this regard, an IL-1-like substance is known to be elaborated by endothelial cells (21), polymorphonuclear leukocytes (22), and vascular smooth muscle (23), all of which are residents of the normal and diseased lung. All of these cell types seem to be involved with monocrotaline-induced pneumotoxicity. Since macrophages also release IL-1, it may be significant that monocrotaline-induced pneumotoxicity is associated with the appearance of an "abnormal" alveolar macrophage, the contents of which increased in parallel with development of pulmonary hypertension (24). It thus seems that a number of potential sources of IL-1 are present in the monocrotaline-treated pulmonary hypertensive lung.

In summary, the present data indicate that a substance with IL-1 bioactivity can be detected in increased amounts in BALF from monocrotaline-treated rats with lung injury and pulmonary hypertension. In addition, accumulation of IL-1 bioactivity appears to be temporally related to development of pulmonary edema and pulmonary hypertension in this animal model. These observations raise the provocative suggestion that IL-1 could play a pathogenetic role in certain forms of lung injury and/or pulmonary hypertension.

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