LOCALIZATION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN A RAT MODEL OF SILICOSIS

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We are participating in a collaborative project at NIOSH on mechanisms of silicosis, in which rats were exposed to filtered air (control) or silica aerosol of 15 mg/m^3 (6 hr/day, 5 days/week), with assays conducted at intervals over a 6 month period.[1] We are especially interested in the role of nitric oxide (NO) in the pathophysiology of lung disease. One source of NO is the alveolar macrophage, known to express the inducible form of nitric oxide synthase (iNOS); we used immunohistochemical techniques to determine whether iNOS is upregulated in alveolar macrophages and other lung sources in response to silica.

Silica-exposed and air control animals were sacrificed after 10, 20, 41, 79, and 116 days of exposure. Formalin fixed, paraffin embedded tissues were cut at 5μm, deparaffinized in xylene and rehydrated. Slides were microwaved in citrate buffer, pH 6.0 for antigen retrieval.[2] After blocking endogenous peroxidase in 3% H_2O_2:Methanol (1:1), slides were placed in 10% BSA for 30 min, then incubated overnight in the primary antibody (Monoclonal anti-iNOS, Transduction Laboratories, N32020, 1:50 dilution). The DAKO LSAB-2 kit for rat specimens (K0609) was used to label the antibody, with diaminobenzidine as the chromagen. Positive controls were lung sections from rats that had been instilled with lipopolysaccharide, and were known to have high iNOS positivity in the alveolar macrophages.

There was very little staining with the iNOS antibody 10, 20, and 41 days after silica or air exposure. At 79 days, however, striking differences were seen. Very little staining was present in the air controls (Fig. A), but in the silica exposed animals there was staining of alveolar macrophages and alveolar epithelial cells that appear to be Type II cells (Fig. B), most prominent in subpleural areas. Intense iNOS staining was also localized in silicotic granulomas (Fig. C) and histiocytic aggregates in bronchial associated lymphoid tissue (BALT) (Fig. D). These localizations were also apparent at 116 days.

In conclusion, we first observed an increase in iNOS in rat lungs after 79 days of silica inhalation. Besides being localized in alveolar macrophages, iNOS is also found in alveolar epithelial cells, silicotic granulomas, and histiocytic aggregates in BALT. These results correspond to other indicators of lung damage seen after silica inhalation in rats.[1,3]

References:
1. Porter D.W., *et al.*, (1999) The Toxicologist 43: In press.
2. Takahashi, A., *et al.*, (1997) Cancer Res. 57:1233
3. Suarez, F., *et al.*, (1999) The Toxicologist 43: In press.

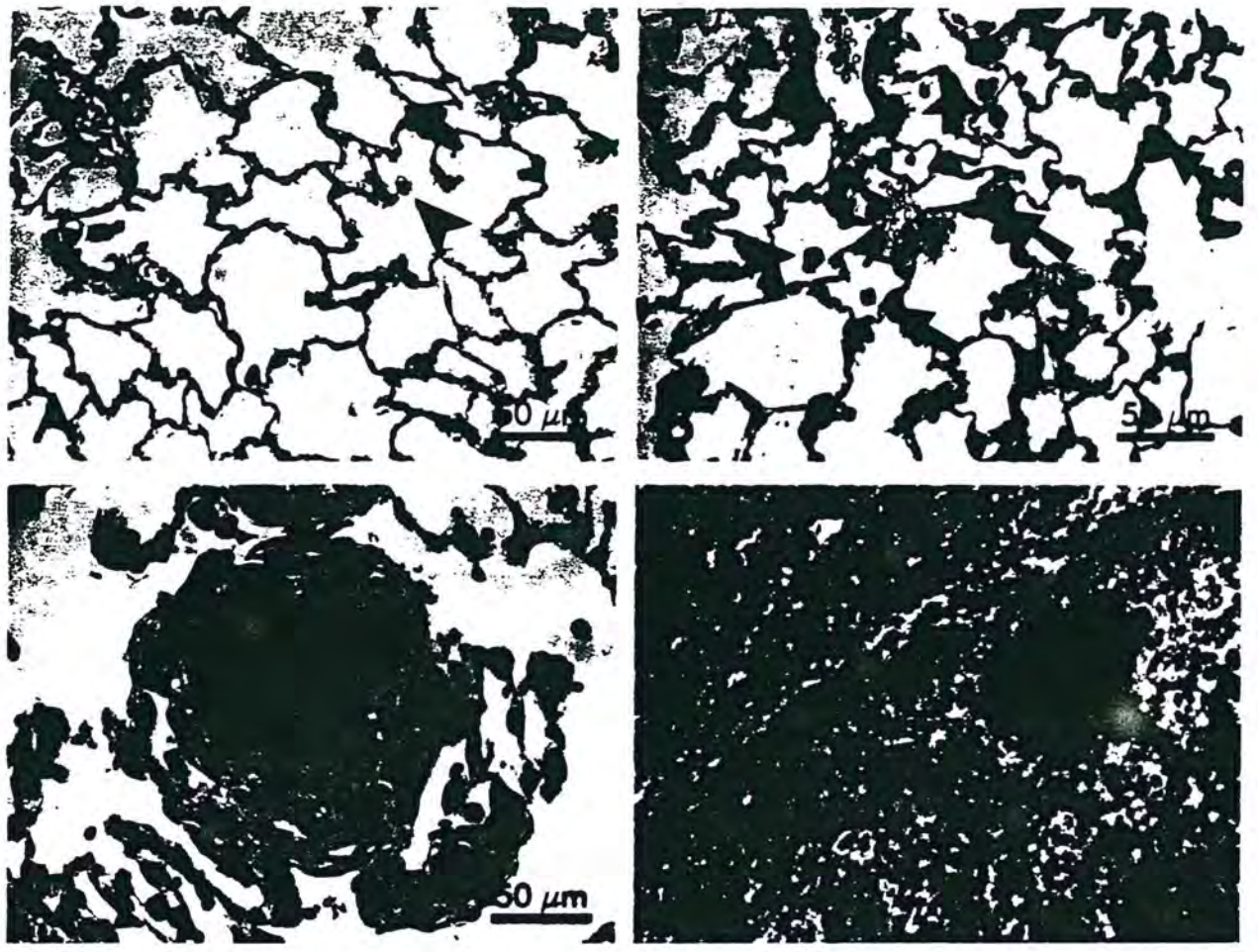

Fig. A. Lung tissue from a control rat exposed to air for 79 days. Immunohistochemical localization of iNOS in the alveolar region. There is no significant staining. Arrowhead - alveolar macrophage.

Fig. B. Lung tissue from a rat exposed to silica for 79 days. Immunohistochemical localization of iNOS in the alveolar region (arrowheads - positive alveolar macrophages; arrows - positive alveolar epithelial cells).

Fig. C. Lung tissue from a rat exposed to silica for 79 days. Intense staining for iNOS (arrow) in a silicotic granuloma.

Fig. D. Lung tissue from a rat exposed to silica for 79 days. Intense staining for iNOS (arrows) within histiocytic aggregates in bronchial associated lymphoid tissue (BALT).

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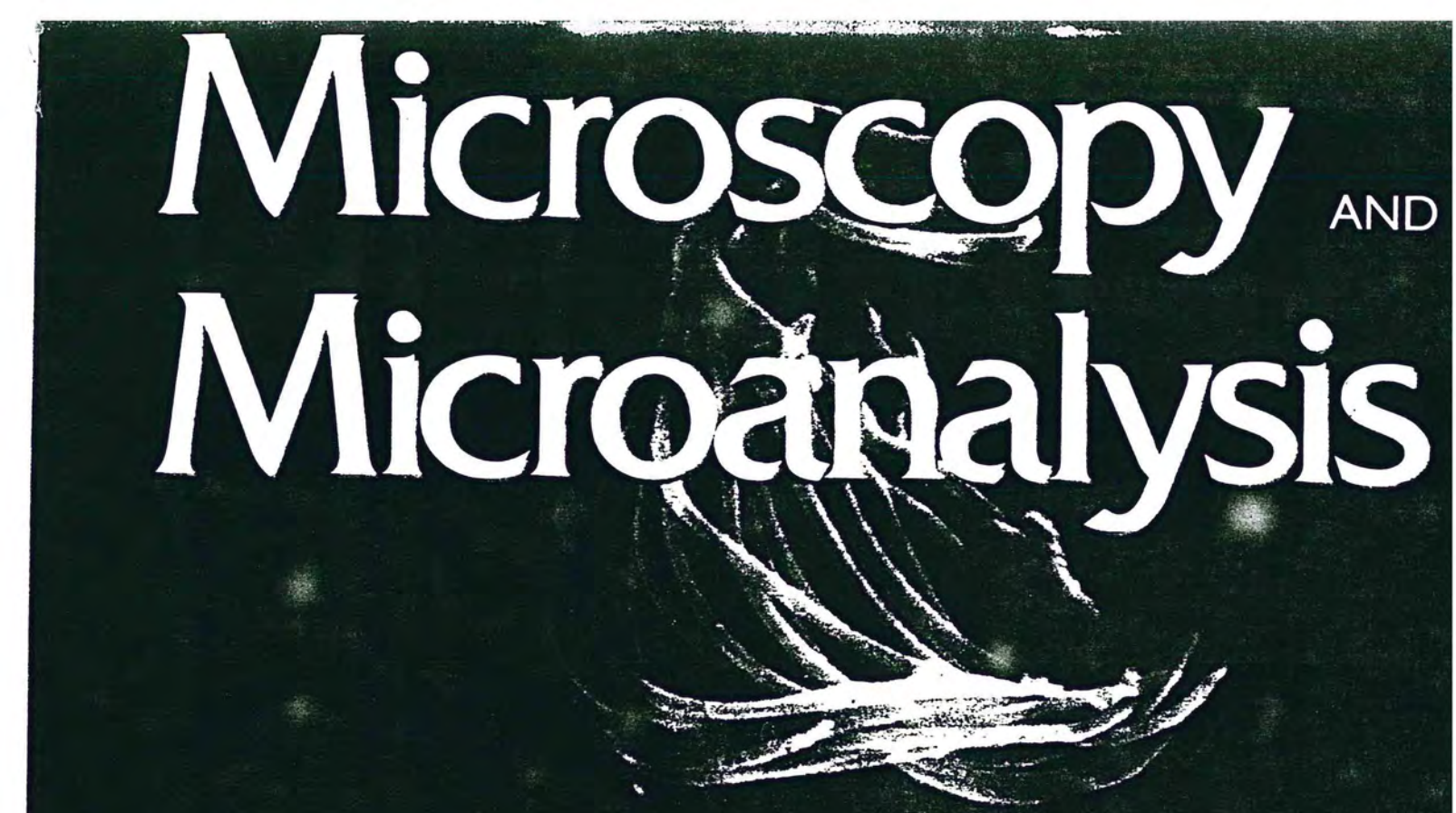

Volume 5, Supplement 2

Proceedings:

Microscopy & Microanalysis '99

Portland, Oregon, August 1–5, 1999

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