Expression and Localization of TIMP-1, TIMP-2, MMP-13, MMP-2, and MMP-9 in Early and Advanced Experimental Lung Silicosis

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INTRODUCTION

Chronic exposure to crystalline silica particles results in macrophage-lymphocytic granulomatous lung inflammation, which is followed by abnormal and progressive accumulation of extracellular matrix.[1,2] However, the pathogenic mechanisms and the sequence of the pathological events leading to the fibrotic response have not been well defined. In this context, there is evidence suggesting an upregulation of a variety of fibrogenic cytokines such as tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β), with increased synthesis and secretion of lung extracellular matrix components.[3,4] However, studies on matrix degradation are scanty.

Extracellular matrix degradation involves the matrix metalloproteinases (MMPs), a conserved family with a zinc binding site in the catalytic domain, and an amino terminal domain responsible for the zymogen inactive state. MMP family include the collagenases, which degrade fibrillar collagens; the stromelysins, which cleave proteoglycans and some glycoproteins; the gelatinases A and B, which degrade basement membrane type IV collagen; and the membrane-type metalloproteinases, which are able to activate progelatinase A.[5,6] MMPs activity is regulated at different levels including the transcriptional level, the proenzyme activation, and the inhibition of active enzymes by a family of tissue inhibitors of metalloproteinases (TIMPs).[7] Here we determined the temporal pattern of expression and localization of collagenase-3 (MMP-13), gelatinases A and B (MMP-2 and MMP-9), and TIMP-1 and TIMP-2 during the evolution of rat experimental silicosis.

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MATERIAL AND METHODS

Lung silicosis was induced in adult Wistar rats by a single intratracheal administration of 50 mg of quartz dust in sterile saline. Eight rats were sacrificed at 15, 45, and 60 days after silica instillation, and eight normal animals instilled with saline were used as controls. Animals were anesthetized, and the lungs were instilled with 4% paraformaldehyde and used for histology, *in situ* hybridization, and immunohistochemistry, as described elsewhere.[8,9] Additionally, bronchoalveolar lavage (BAL) was performed in six controls and six silica-exposed rats at 15, 45, and 60 days, and aliquots of 8 µl of fluid were used to analyze gelatinase activity in gelatin substrate SDS gel as previously described.[8]

RESULTS

A significant increase in total inflammatory cells was observed in BAL from silicotic rats. The inflammatory response was characterized by an increment of lymphocytes and neutrophils at 15 and 45 days, and also by macrophages at 60 days. Zymography of BAL fluid from silica-exposed rats revealed increased gelatinolytic activities of progelatinase A and its activated form when compared with controls. Additionally, silicotic rats also showed bands with ~ MW of 95 and 86 kDa representing progelatinase B and its activated form.

By *in situ* hybridization and immunohistochemistry, younger silicotic granulomas exhibited intense staining for MMP-2, MMP-9, MMP-13, TIMP-1, and TIMP-2. Labeling was usually restricted to the granulomas and surrounding areas. By contrast, older granulomas, characterized by the presence of concentric layers of hyaline fibers in the center, displayed similar staining for TIMPs, but MMP signaling was markedly reduced. Saline-treated animals showed scattered positive cells. A semiquantitative evaluation is shown in TABLE 1.

Collagenase-3 transcript and protein was detectable in alveolar epithelial cells, macrophages, and fibroblasts. MMP-2 mRNA was observed mainly in mesenchymal cells and macrophages, while MMP-9 mRNA was expressed by macrophages, type 2 pneumocytes, and neutrophils. TIMP-1 and TIMP-2 were expressed by macrophages and mesenchymal cells.

TABLE 1. Semiquantitative evaluation of MMPs and TIMPs expression during the evolution of silicosis

Time after silica exposure	MMP-13	MMP-2	MMP-9	TIMP-1	TIMP-2
15 Days	+++	+++	++	+++	+++
45 Days	++	++	++	+++	+++
60 Days	+	+	+	++	+++

DISCUSSION

The findings of this study suggest that in early inflammatory silicotic granulomas there is a marked upregulation of collagenase-3 and gelatinases A and B, which decrease when the lesions evolve to fibrosis. By contrast, TIMP-1 and TIMP-2 also display a considerable increase from the early phases but show a more discrete reduction in the fibrotic granulomas. These results support the notion of an imbalance in the MMP/TIMP ratio during the evolution of experimental silicosis that could enhance the fibrotic response. Excessive initial gelatinolytic and collagenolytic activities may participate in basement membrane disruption, matrix remodeling, and growth factor release. Decreased collagenolytic activity in advanced phases may contribute to collagen accumulation and the development of progressive fibrosis.

ACKNOWLEDGMENTS

This work was supported by PUIS & PAPIT: IN202697 (UNAM); CONACYT F643-M9406.

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