Bleomycin injury of the lung in a mast-cell-deficient model

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Abstract

Lung mast cell hyperplasia and fibrosis is induced by bleomycin lung injury. The role of the mast cell in this process of injury and resultant fibrosis is unclear. Mutant mi/mi mice, profoundly mast-cell-deficient, were treated with intraperitoneal bleomycin and demonstrated minimal acute inflammatory and chronic fibrotic responses. Lung histamine values determined at 14 and 42 days after bleomycin injury in mi/mi mice were not increased compared to untreated mi/mi animals. However, lung histamine levels in normal mice demonstrated a 300% increase over controls on Day 14 after bleomycin injury, and then returned to baseline by Day 42. The mi/mi BAL cell recovery at 2 weeks after injury and lung hydroxyproline levels at 4 weeks after injury were not altered from baseline. The normal litter mates, in contrast, demonstrated significant increases compared to controls in both of these parameters (p < 0.01, p < 0.04). Although the mi/mi mouse is also deficient in basophils, natural killer cells and functional osteoclasts, there is no evidence of lowered pulmonary defense mechanism and neutrophils and alveolar macrophages are present in normal numbers. This investigation supports the hypothesis that the mast cell contributes to bleomycin-induced lung injury and fibrosis.

Introduction

Bleomycin sulfate is an antineoplastic antibiotic that induces pulmonary fibrosis in both humans and experimental animals. Cellular mechanisms of bleomycin-induced pulmonary fibrosis in experimental animals have been intensively studied as a model of human pulmonary fibrosis [1–3]. The pulmonary mast cell is known to contain a variety of mediators that can contribute to lung inflammation and may also modulate fibroblast function [4–6]. Histologic studies of lung tissue demonstrate a significant increase in the number of pulmonary mast cells following bleomycin injury associated with an increase in lung histamine concentrations [7]. The role of mast cell products in lung injury and subsequent fibrosis induced by bleomycin has not been evaluated. In this study we investigated the possible contribution of mast cell products to bleomycin-induced lung injury by utilizing a previously characterized mast-cell-deficient C57JB1/6J mouse animal model. Mutant mice of mi/mi genotype have only 0–10% of the normal mast cell density depending on tissue studied and are deficient in basophils, natural killer cells and functional osteoclasts [8]. Mast-cell-deficient mice (mi/mi) and normal homozygote (+/+) litter mates were treated with intraperitoneal
bleomycin and subsequently, the degree of early injury was evaluated by quantitating the inflammatory cells recovered by bronchoalveolar lavage, and the subsequent degree of lung fibrosis was evaluated by determining total lung hydroxyproline content. Histamine levels were measured to evaluate the presence of mast cells. The mi/mi group studied at 14 days was also evaluated by histopathological examination.

Methods

Animal model

Osteopetrotic mast cell deficient mi/mi mice and normal litter mates were obtained from the Kansas City Veteran's Administration Medical Center's Calcium Research Laboratory. These animals were descendants of C57Bl/6J +/mi breeders originally donated to the laboratory by Dr. Don Walker. With this genetic background, the normal litter mate (+/+), and mi/mi mice can easily be distinguished from one another because an mi/mi mouse is a microphthalmic pseudo-albino. All animals were weighed weekly and kept in the same environment. The animals were given food and water ad libitum.

Bleomycin

Bleomycin sulfate (Blenoxane, Bristol Laboratory, Rochester, New York) was dissolved in sterile 0.9% saline and injected intraperitoneally. The concentration of bleomycin was 1 µg/100 µl and the volume injected was approximately 100 µl/20 g of murine body weight.

Treatment regimens

Two treatment regimens with bleomycin were used to evaluate the early and late pulmonary response to bleomycin injury. Group 1: 13 mi/mi mice and 13 +/+ litter mates were treated with intraperitoneal bleomycin in six doses of 50 µg/kg over 14 days for a cumulative dose of 300 µg/kg. This treatment regimen resulted in a mortality of 23% in both groups. The surviving animals in each group were killed on Day 42 and the lungs were removed for hydroxyproline determination as well as histamine determination. Five mice in each group were untreated and served as controls.

Group 2: 15 mi/mi and 15 +/+ mice received intraperitoneal bleomycin in three doses of 50 µg/kg over 7 days for a cumulative dose of 150 µg/kg. There was no mortality associated with this treatment in any experimental animal. Animals in the experimental groups were killed on Day 42 and the lungs were removed for hydroxyproline determination as well as histamine determination. Five mice in each group were untreated and served as controls.

Bronchoalveolar lavage

The animals in Group 1 were killed at 14 days and unilateral lung lavage was performed. The animals were anesthetized with intraperitoneal phenobarbital (Nembutal, Abbott Laboratories, Chicago, IL) and killed by exsanguination. The trachea was secured with a ligature and the heart and lung structures were removed from the thorax. The trachea was cannulated with a 20 g catheter that was secured with a ligature and lavage of the right lung was performed using a 1 ml aliquot of saline (0.9% NaCl at room temperature) with a total of four lavages being performed. In all animals studied, recovery of lung lavage fluid was 70% or greater. Recovered fluid was centrifuged at 800 x g for 10 min to sediment the cells. Total cell recovery was determined by hemacytometer and cell viability determined by trypan blue exclusion. Determination of the differential cell recovery was performed by counting 200 cells of a Wright Giemsa stained cytocentrifuge slide preparation.

Measurement of lung hydroxyproline content

Animals in Group two were sacrificed at 42 days. The right lung was surgically removed from each animal and proximal airway structures were also removed. Lungs were homogenized in cold 10% trichloroacetic acid, hydrolyzed with 6 N HCl and lyophilized. Lung hydroxyproline content was determined by amino acid analysis as previously described [9]. Results were normalized to whole lung wet weight.

Histamine assay

Lung tissue was weighed and boiled in saline to extract histamine. Histamine levels were determined by the enzymatic isotopic method [10].