N-Acetyl-Beta-D-Glucosaminidase Activity within BAL from Macaques Exposed to Generic Coal Dusts

P. A. Mack, 1 J. W. Griffith, 2 S. Riling, 2 and C. M. Lang 2

1 Tulane Regional Primate Research Center, 18703 Three Rivers Rd., Covington, LA 70433, USA; 2 Department of Comparative Medicine, Milton S. Hershey Medical Center, Pennsylvania State University, P.O. Box 850, Hershey, PA 17033, USA

Abstract. N-acetyl-beta(\(\beta\))-D-glucosaminidase is a lysosomal enzyme secreted by alveolar macrophages in response to phagocytosis of particulate material. Alveolar macrophages participate in the degradation and fibrosis of pulmonary tissue that results in pneumoconiosis. Known quantities of four characterized respirable dusts were bronchoscopically placed into the right caudal lung lobe of macaque monkeys. Bronchoalveolar lavage (BAL) samples were collected from dust-exposed right lung and unexposed left lung of the same individuals at 2-week intervals for 12 weeks after dust instillation. The samples were tested for N-acetyl-\(\beta\)-D-glucosaminidase activity to determine if the enzyme levels could serve as an indicator of pulmonary injury induced by generic coal dusts when compared to known fibrogenic and nuisance dusts. Installation of generic quartz, anthracite, or TiO\(_2\) dusts produced significant elevations of enzyme activity and increased numbers of macrophages in the dust-exposed lobes. Elevations in enzymatic activity and macrophage numbers were greatest in response to generic quartz dust. These results suggest that quantitative levels of N-acetyl-\(\beta\)-D-glucosaminidase activity may be a useful indicator of acute and chronic lung injury following exposure to fibrogenic and nonfibrogenic dusts.

Key words: Pneumoconiosis—N-acetyl-\(\beta\)-D-glucosaminidase—Bronchoalveolar lavage fluid (BALF)—Disease models—Animal macrophages.

Introduction

Coal workers pneumoconiosis (CWP) is an occupational pulmonary disease associated with the inhalation of coal mine dust. An important external factor in the development of CWP is the composition of the respirable coal mine dust. A cause-and-effect relationship exists between coal mine dust and CWP, but it
has not been shown that exposure to generic coal dusts will cause CWP [12]. Numerous immunological, biochemical, and cellular changes occur in CWP; however, in this study, only N-acetyl-beta(13)-D-glucosaminidase (NAGase), protein, and alveolar macrophages (AM) will be examined [7, 32, 33].

NAGase is secreted by AM in response to phagocytic stimuli [1, 5, 21, 32]. NAGase levels have been previously examined in bronchoalveolar lavage (BAL) specimens from humans and nonhuman primates exposed to coal dust and were useful in distinguishing dust-exposed (fibrotic) lung from unexposed (normal) lung [9, 18, 30]. Humans with CWP had higher NAGase levels and AM numbers than control subjects [30]. NAGase has been reported to be one of the most useful parameters for characterizing the acute inflammatory response to lung injury [5, 11, 21, 24, 33]. It may also be predictive of chronic lung responses [20, 33].

The primary purpose of this study was to determine if NAGase activity would be useful to study the response of dust-exposed and unexposed lung lobes from the same individual following controlled dust exposure. This model is unique since a known quantity of characterized dust is instilled into a local area of one lung. BAL samples are obtained from the dust-exposed area, as well as from an unexposed area of the other lung of the same individual. The use of one lung lobe as a control has been previously documented [2, 3, 15, 16]. The only experimental variable that influenced the outcome of individuals in this study was dust type. This variable was used to compare the cellular and enzymatic response of a known characterized fibrogenic dust to the cellular and enzymatic response of a known characterized nonfibrogenic dust in this model. The second purpose of this study was to characterize further cellular and enzymatic response to generic anthracite and bituminous dusts whose fibrogenic potential is not known.

NAGase was chosen as the enzymatic parameter since it has been shown to correlate closely with acute lung injury [21]. It has also been reported to be a direct and indirect initiator of lung injury and is secreted from AM activated by phagocytosis [5, 21]. AM levels were examined as the cellular marker since they are the primary cell type see histologically in CWP [5, 7, 15, 26, 36].

Materials and Methods

Twelve female pigtail macaque monkeys (Macaca nemestrina), 4.5-5.4 kg weight, were used for dust instillation and repeated BAL sampling. They were individually housed and were on a 12-hour light/12-hour dark cycle with no twilight. The animals were kept at 72 ± 2°F with a humidity of 40-60% and 13 ± 2 complete air changes per hour. They were fed commercial monkey chow with fresh fruit supplementation and were provided fresh water ad libitum. Preventive veterinary care was provided as part of an AAALAC accredited laboratory animal facility.

The animals were fasted 12-24 hours prior to anesthesia, which was produced with ketamine at a dosage of 10-25 mg/kg. Three BAL were performed at 2-week intervals prior to dust instillation in the right lung for comparison to the left (control) lung after dust instillation [2, 3, 15, 16]. The dusts were instilled into the right caudal lung lobe using a flexible fiberoptic bronchoscope. After dust instillation, BAL was performed once every 2 weeks for 12 weeks. For dust instillation and BAL into the right lung, the bronchoscope was advanced through the oral cavity into the trachea and gently wedged in the bronchi of the right caudal lobe. For the BAL, sterile PBS was infused into the right caudal lung lobe in two 50-ml aliquots (total of 100 ml). Each 50-ml aliquot was then