EXPOSURE TO GRAM-NEGATIVE BACTERIA AND THE DEVELOPMENT OF BYSSINOSIS

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ABSTRACT

Many studies have indicated a relationship between exposure to cotton dust contaminated with bacteria and the development of respiratory symptoms. Samplings of cotton products from different geographical locations have shown a contamination with Gram-negative bacteria which is particularly high in the stem and bract portion of the plant. Cultures of bale cotton all demonstrate bacterial contamination, predominantly with Gram-negative bacteria.

A series of animal experiments was undertaken to elucidate the mechanism behind the development of byssinosis. An acute exposure to extracts from cotton dust produced an increase in the number of polymorphonuclear leukocytes in the airways of animals 24 hours after exposure. The magnitude of the increase was related to the number of Gram-negatives in the different dusts studied. In subacute experiments, the number of leukocytes in the airways remained elevated but decreased rapidly at cessation of the exposure. A renewed exposure 3 days afterwards caused an increase in the number of leukocytes.

In animals subacutely exposed to an aerosol of endotoxin, a bronchial immune response, mainly related to the IgA class of immunoglobulins, developed rapidly. Following cessation of exposure and re-initiation after 3 days no alterations in the titres of anti-endotoxin IgA could be found. A correlation was found between the ratio of IgG/IgA antibodies and leukocyte number in the airways.

The results suggest that "Mill fever" is caused by an acute reaction to endotoxins to which tolerance develops and that byssinosis is related to a leukocyte migration into the lungs from the blood. The continued presence of leukocytes in the airway epithelium with secretion of hydrolytic enzymes could explain the later development of chronic bronchitis.

The possible role of bacteria for the development of byssinosis has been recognized for several decades. Observations and reports from 1936 and 1942 give evidence of a connection between Gram-negative bacteria and pulmonary symptoms in cardroom workers and other categories of workers exposed to contaminated cotton. Pernis and co-workers reported on basic animal work using endotoxins as the agent. Airborne Gram-negative were measured in cotton mills by Cinkotai and co-workers and Rylander and co-workers.

This paper reports data from animal experiments designed to investigate responses in the respiratory tract after exposure to an aerosol of cotton water.
extract, Gram-negative bacteria or their endotoxin (lipopolysaccharide, LPS). The aim of the experiments was to relate the observed changes in the respiratory tract to the clinical symptoms demonstrated by exposed cotton mill workers.

MATERIAL AND METHODS

Full grown guinea pigs and rats were used in the experiments. They were exposed to an aerosol of bale cotton water extract, cotton dust extract, Gram-negative bacteria isolated from cotton or endotoxin (LPS). The exposure took place in a stainless steel chamber where the agent to be studied was aerosolized with a Collison spray\textsuperscript{11}. The animals were acutely exposed for 40 minutes once each day for 10 days. In some animals the exposure was resumed after one day to create the same situation as in workers returning to work after the weekend.

At the time of the examination the animals were killed with an overdose of sodium pentothal i. p. A lung lavage was performed, a sample of the fluid stained and the number and types of free lung cells were counted. Determinations of antibodies to LPS in serum and the bronchial washings were made with the ELISA technique\textsuperscript{2}.

RESULTS

The basic response occurring in the animal lung after exposure to water extract of cotton, Enterobacter or LPS is an increase in the number of polymorphonuclear leukocytes (PMN). This increase begins a few hours after the exposure and reaches a maximum level at about 24 hours. Thereafter a gradual decrease in the number takes place. When extracts from cotton dusts or bale cotton from different factories were tested the increase was related to the number of Gram-negative bacteria in the different preparations\textsuperscript{12}. In animals given continuous exposures, the number of polymorphonuclear cells in the bronchial fluid declined following the first peak 24 hours after exposure but remained elevated in comparison with controls. When the exposure was interrupted after 10 days, the number of leukocytes decreased to the values of the controls after 3 to 4 days. A renewed exposure brought about a rapid increase again\textsuperscript{13}.

Determinations of antibodies to LPS in the bronchial fluid failed to demonstrate the presence of IgG, IgA or IgM anti-LPS antibodies in control animals. Among the exposed animals practically all had IgA antibodies\textsuperscript{14}. IgG antibodies were found in about 30% of the animals and IgM antibodies only in two out of 60 animals.

In serum IgA and IgM antibodies were present in about 30% and 40% respectively of the animals and IgG antibodies in about 70%. In the animals whose exposure was stopped for 3 days and then re-initiated, no significant alterations in antibody levels could be detected, either in serum or in the bronchial fluid.

The relationship between the number of polymorphonuclear leukocytes and the antibody levels in the bronchial fluid was analysed. It was found that the number of leukocytes was significantly correlated to the IgG/IgA ratio in the bronchial fluid (p < 0.02, Student's t-test).
DISCUSSION

The results obtained in the animal studies demonstrate that the same cellular response occurs in the airways when extracts from bale cotton, Gram-negative bacteria or LPS are inhaled. A rapid increase in the number of PMN takes place probably by migration through the capillaries. An increase in the number of polymorphonuclear leukocytes on the airway epithelium after exposure to cotton dust or endotoxin has also been found in other animal models and in cardroom workers. This invasion in the lung of PMN represents the first stage of an acute inflammatory reaction.

The data also show that after repeated exposures, a local immune defense develops chiefly in terms of IgA antibodies. The IgG antibodies that were found in the airways could be locally produced, but it is more likely that they originate from the serum, as the correlation between IgG antibodies in the bronchial fluid and serum was significant.

The levels of antibodies in the airways did not change during the break in exposure, whereas the number of PMN fell to normal levels and increased as soon as the exposure was started again.

Provided that the observations in animals basically correspond to the reaction taking place on humans, it is suggested that the clinical symptoms reported by workers in cotton mills after a break in the exposure over the weekend are not related to fluctuations in the antibody levels in the airways or in the serum, but to alterations in the size of the population of PMN in the lungs.

It is tempting to speculate that the agglomeration of leukocytes in the capillary vessels on their way into the lung increases the blood pressure in the pulmonary veins and provokes subjective symptoms of chest tightness. This reaction has been demonstrated in other animal models. Further research has to be undertaken, however, before this reaction can be identified as the explanation for byssinosis.

The reason for the correlation between leukocytes and the IgG/IgA antibody ratio in the bronchial fluid is not known. It is conceivable that a higher proportion of IgG antibodies in the airways causes an increased formation of immune complexes when LPS is inhaled. If these complexes have a higher biological potency, for example by activating complement, this could lead to a release of leukotactic compounds and an increased number of PMN. Experiments are in progress to test this hypothesis.

ACKNOWLEDGEMENT

The work reported here has been supported by a grant from Cotton Inc. Raleigh, N.C., U.S.A.

REFERENCES


