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Lymphadenitis as a major element of disease in the guinea pig model of tuberculosis

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Summary Guinea pigs infected by low dose aerosol with the H37Rv strain of *Mycobacterium tuberculosis* rapidly developed granulomatous lesions in the pulmonary parenchyma and within the intra-thoracic hilar lymph node cluster. Lung lesions showed no predilection for specific lobes and were perivascular, peribronchial and peribronchiolar throughout the subpleural, hilar and pulmonary parenchyma. Marked hilar lymph node enlargement was due to coalescing foci of subcapsular, paracortical and medullary granulomatous inflammation that progressed to necrosis that effaced normal lymph node architecture. Lymph node lesions became severe and progressed more rapidly than pulmonary lesions. Immunization with BCG 6 weeks prior to infection significantly reduced the lung and lymph node lesion burden as well as the progression to necrosis in both tissues. Lymph node inflammation in BCG immunized animals partially resolved and was replaced by fibroblasts and fibrous connective tissue while lesions from non-immunized animals continued to progress to necrosis. We discuss here the observation that the distribution and progression of lung and lymph node lesions in the guinea pig aerosol model of tuberculosis have considerable similarity to the naturally occurring disease in children.

Introduction

The number of cases of tuberculosis in children is on the rise both in developed and developing countries.[1–7] The WHO has estimated that among the approximately 1.3 million annual cases of

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pediatric tuberculosis in developing countries, 450,000 deaths were among individuals younger than 15 years of age. Of the estimated 8.3 million new cases of tuberculosis world wide in the year 2000, approximately 10% occurred in children.[4] Because tuberculosis in children usually reflects recent infection through close contact with infected adult parents, the frequency of new pediatric cases has been considered a sentinel for failed tuberculosis control programs in adults.[6,8]

The importance of tuberculosis in children is in the apparent differences in pathogenesis compared to adults and the difficulty in early diagnosis and treatment.[9–11] In infants from birth to children 4 years of age, clinical tuberculosis is rapidly progressive with early extra-pulmonary spread that can result in the more lethal forms of the disease. As in adults, the primary site of infection in children is the lung, but rapid dissemination of bacilli can result in systemic spread to multiple organs.[9,12] Among the most common sites of extra-pulmonary spread are the intra-thoracic hilar lymph nodes and the meninges of the brain and spinal cord.[5,9] Delayed or failed treatment of these manifestations of tuberculosis in children can result in death from acute airway restriction or severe and permanently disabling CNS or spinal disease.

BCG is the only widely accepted vaccine used in the prevention of tuberculosis. Despite the widely disparate reports on efficacy of BCG in preventing tuberculosis, it is generally accepted to be most effective in preventing or lessening the severity of pediatric disease.[13,14] Recent studies suggested that BCG reduces the risk of extra-pulmonary dissemination of bacilli and thus reduces the incidence of milliary, meningeal and osseous tuberculosis when given to newborns subsequently infected or exposed to *M. tuberculosis* from birth to 4 years of age.[15,16]

In a recent study our laboratory developed a magnetic resonance imaging (MRI) technique to visualize lesions in the lungs of guinea pigs infected by low-dose aerosol of *M. tuberculosis*. In that study we recognized a rapid and progressive enlargement of intra-thoracic hilar lymph nodes that was due to rapidly progressing granulomatous lymphadenitis.[17] We noted that involvement of hilar lymph nodes following low-dose aerosol challenge in the guinea pig resembled the early, extra-pulmonary manifestation of tuberculosis seen in children. Accordingly, the purpose of the present study was to further characterize the temporal relationship between the emergence of lesions in the lung and lesions developing in the intra-thoracic hilar lymph nodes, including the potential protective effect of BCG vaccination. The results of this study show that granulomatous inflammation and necrosis of intra-thoracic hilar lymph nodes is an early and significant manifestation of low dose aerosol infection of guinea pigs with *M. tuberculosis*.

Methods

Experimental infections

Female outbred Hartley guinea pigs (~500 g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA, USA) and held under barrier conditions in a Biosafety Level III animal laboratory. *M. tuberculosis* H37Rv and *M. bovis* BCG Pasteur were grown from low-passage seed lots in Proskauer–Beck liquid media containing 0.05% Tween 80 to early mid-log phase. Cultures were aliquoted into 1-ml tubes and frozen at −70 °C until used. Thawed aliquots were diluted in double-distilled sterile water to the desired inoculum concentrations. A Madison chamber aerosol generation device was used to expose the animals when they were approximately 5 months old to an aerosol of *M. tuberculosis* and was calibrated to deliver approximately 20 bacilli into the lungs.

Tissue fixation and processing

Lungs were harvested from control and vaccinated guinea pigs at various times after exposure to infection with *M. tuberculosis*. At the time of euthanasia, lungs were inflated with approximately 60 ml/kg of room air by tracheal intubation. The pulmonary vasculature was flushed of blood with 45–60 ml of phosphate buffered saline (PBS) and perfusion fixed with 45–60 ml of 10% neutral buffered formalin (NBF) via the right ventricle. Following perfusion fixation, lungs were removed en bloc and immersion fixed overnight in 10% NBF. After overnight fixation, heart, adjacent vessels and esophagus were dissected from the lungs, and the surface dried. The lungs and associated mediastinal lymph nodes were embedded in 4% low melting point agarose in PBS. Embedded lungs were sectioned into approximately 18–24 2-mm slices using an aluminum template. Furthermore, one section containing a portion of the left caudal lung lobe was taken and processed normally for histological evaluation after staining with hematoxylin and eosin.

Lesion analysis

To evaluate the concurrent progression of lung and lymph node lesions a histological grading system