AN ASSESSMENT OF ALVEOLAR MACROPHAGE ACTIVITY IN DOGS

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Introduction

By functioning as scavengers, the alveolar macrophages (AM) act as major defenders of the lower respiratory tract against potentially noxious inhaled particles. Furthermore the AM as those of their members of the mononuclear phagocyte system, play a role as accessory cells in immunologic responses. Samples obtained by pulmonary lavage have enabled studies to be made of humoral and cellular elements of the lower respiratory tract of humans'. Likewise, several infectious or non-infectious respiratory diseases of dogs can be used as models for their potential use as animal models and comparative studies. The purpose of this study was to report a modified method of isolating AM via bronchiolar-alveolar lavage in dogs with the following objectives; to assess the AM phagocytic and intracellular killing activities.

Materials and Methods

Two dogs obtained from the Dog Unit, Dewan Bandaraya Kuala Lumpur were acclimatised for a week. The first dog was a clinically healthy adult (A) while the other was a young puppy with evidence of respiratory disease (Y). After euthanising the dog with barbiturate, the entire ventral part of the body was surgically prepared. The lung was plucked and the trachea clamped at the area close to the larynx. After the lung were assessed for the presence of gross abnormalities, fifty ml of warm PBS was poured into the tracheal lumen and the entire lung was massaged for 30 seconds. The resultant lavage was then poured into a sterile beaker covered with four layers of sterilised cotton gauze. The lavage was then centrifuged three times at 300g at 4°C for ten minutes. The supernatant was then collected for the assay of AM activity using the acridine orange cheluminescence assay. In order to assess the validation of the AM activity, the same set of samples were reassayed the next day. Results obtained were subjected to paired t-test (SPSS).

Results and Discussion

The lung of A was within normal limits except for the presence of anthracosis, which was also seen in Y. A dark red firm consolidated area was found in the apical lobe of lung Y. In addition to the lavaged PBS approximately fives ml of mucopurulent exudate was also retrieved from lung Y. The acridine range chemiluminescence assay proved to be a very reliable indicator for the quantification of AM activity. The fact that was demonstrated from the lavage in this study confirmed that the fluid did reach the alveoli. The phagocytic and intracellular killing activities for A and Y at Day 1 and Day 2 is 63.3±8.1 and 37.3±2.1, and 78.1± 2.1 and 40.9±8.7; and 69.3±0.6 and 64.7±4.0, and 84.5±2.2 and 57.6±4.6, respectively. The technique used in this study yielded comparable AM in both dogs. It appeared that AM phagocytic activity remained constant irrespective of age status or health status. Exhaustive literature search indicated that research activities were based on normal dogs and cat.Thus it is rather difficult, from this short study, to make an assumption that this parameter remains unchanged despite of age or health status. However, a possible explanation can be deduced by considering measurement of the activity at Day 2. There was a marked reduction in AM phagocytic activity in A while there was no change in Y. It is possible that in Y, part of the lung was infected and the AM were sensitised by the inflammatory reaction and thus able to perform their function even after 48 hours. The intracellular killing activity, however, was insignificantly higher in Y. Nevertheless, the killing ability of AM in both A and Y declined rapidly after day 2, albeit still insignificantly higher in Y. This finding indirectly supports the suggestion that both AM activities measured in this study are greater in Y. The insignificance was due to small sample size and small lung lesions. Likewise, in Y the exudate was mainly mucopurulent indicating a subacute and possibly bacterial infection. Thus, if a Giemsa stain was done, it may show greater percentage of neutrophils. It was suspected, in this study, that the entrapment by the exudate might have prevented complete elution of AM.

Conclusions

This technique is comparable to established techniques described earlier such as the bronchial brushing and punch biopsies surpass those commonly used technique of Giemsa staining. In addition, this staining method is unable to demonstrate the intracellular killing activity. The result of the study reported here has great relevance both as a diagnostic and research tool in human and animals especially in the field of environmental and respiratory pathology.

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