would suggest that larger studies need to be undertaken and explored for confounding factors such as smoking before a statement can be made that rheumatoid factor is protective against honeycomb.

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References

Measuring granulocyte apoptosis in airway inflammation

We read with interest the paper by Turlej et al describing enhanced survival of lung granulocytes in an animal model of asthma.1 As discussed by the authors, modulation of immune cell apoptosis is likely to be important in controlling inflammatory processes, and the paper enhances our understanding of this.

However, we feel that there are some methodological problems with the study. Firstly, the animal model they describe, though having some similarities with asthma, is closer to chronic obstructive pulmonary disease. Neutrophils are the predominant inflammatory cells in this model. This condition is often known as COPD in horses.1

Secondly, although the authors refer to the use of annexin V (AV) and propidium iodide (PI), they do not describe the methodology used or how they interpreted the staining with AV and PI. This is important because there are controversies surrounding the interpretation of this method of assessing apoptosis.2 The interpretation of the various staining patterns is controversial. In addition, at least two methods should be used to confirm apoptosis,3,4 and only one is used in the study.

It is noted that the blood granulocytes are isolated by use of a density gradient. Density gradients may interfere with some neutrophil functions5 and this must be borne in mind when interpreting these results. Additionally, BAL granulocytes from healthy horses were isolated by use of a density gradient, whereas BAL granulocytes from healthy horses have apoptotic rates of around 40%. This difference of methods introduces a potential bias into the study. We have previously attempted to isolate neutrophils from human BAL fluid with no success (unpublished observations) and would be interested to know if the authors achieved this separation easily. We are also surprised at the viability of >90%. Cell viability is likely to diminish with increasing rates of apoptosis, and it is notable that the BAL granulocytes from healthy horses have apoptotic rates of around 40%.

This study is interesting, but the methodological issues raised must be considered in interpreting the results.

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References

Authors’ reply

We thank Dr Kelly and colleagues for their interest in our paper. In the past equine heaves was called COPD but, because equine heaves is completely different from human COPD, specialists in the field have recommended avoiding the erroneous term “COPD” for designating this disease. Indeed, it is now clear that equine heaves is very close to atopic asthma and these diseases share important characteristics features including hypersensitivity to aeroallergens, Th2 type immune response, chronic airway inflammation, reversible airway obstruction, non-specific airway hyper-responsiveness, and production of eosinophils specific IgE. It is correct that neutrophils are the predominant inflammatory cells in equine heaves, but this does not exclude the use of this model in asthma studies. Indeed, neutrophils are known to play an important role in asthma whereas recent studies have questioned the importance of eosinophils in this disease.4,5

In our study only small amounts of granulocytes were recovered from the lung of the horses so we were only able to use one method to assay these cells for apoptosis. We chose the method that has been found to be the most sensitive marker of granulocyte apoptosis—the annexin V (AV)/propidium iodide (PI) method.7 The results obtained with this method were interpreted as follows: AV+/PI− cells were considered alive, AV+/PI− cells were considered apoptotic, and AV+/PI+ cells were considered necrotic. This is the first time we have heard of controversy surrounding the interpretation of the results obtained with this method, probably because they have not been published in scientific journals. According to the archives we have read using the web addresses provided by Dr Kelly and colleagues, it appears that this controversy exclusively concerns the status of AV+/PI+ cells. Such cells are uncommon and were not observed in our study.

We agree that density centrifugation may interfere with neutrophil function. To the best of our knowledge there is no other way of separating granulocytes from other cell types. As mentioned in the Methods section of our paper, cell viability of freshly isolated granulocytes was evaluated by trypan blue (TB) exclusion. The cells were then cultured for different times and assayed for apoptosis using AV/PI. Cells in an early state of apoptosis are AV+ and TB+. It is surprising to find 40% apoptotic (AV+) cells in a population where nearly all the cells (>90%) are TB−.

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References

CORRECTION

In the Programme and Abstracts of the British Thoracic Society Winter Meeting 2001 published in Thorax 2001;56(Supplement III), an error occurred in abstract S30 “Management of pneumothorax in a district general hospital” in compliance with the BTS guidelines” by Al-Aloul M, et al which appeared on page iii40. The name of the second author which appeared as K U Torrey should have been K U Toor.