Matters arising

Dr Fox et al comment: Dr Damiano points out that our classification of emphysema was inadequate. We felt, however, that it was important to measure emphysema in the same tissue sections used for immunogold analysis, thus the tissue we examined for localisation of human neutrophil elastase (HNE) was that on which we had measured transect lengths. To eliminate the high background staining that we observed with formalin, we fixed the tissue for electron microscopy by a modified Karnovsky method. We then went on to measure transect lengths; this is the distance between alveolar septa as measured on random lines by computer image analysis; it is not the same as the mean linear intercept (MLI) described by Dunnill[1] and used by Damiano et al.[2]

Thus both the preparation of the tissue and the method by which the tissue was measured were different to the methods used by Damiano et al. When we compare the degree of emphysema, however, (fig 1) with that shown by Damiano et al (fig 7) as severe emphysema, our cases seem comparable with those described by Damiano et al. Therefore, we do not think that we have worked with tissue showing minimal emphysema, and furthermore, in measuring transect lengths and immunogold label in the same section, we are confident that the tissue we labelled with immunogold was emphysematous. We remain interested to know how Damiano et al knew that the sections they labelled with immunogold were emphysematous.

We did not describe in detail the conditions under which we were able to get positive binding of gold-labelled antibody to elastin in the absence of primary antibody; this entailed longer incubation times, possibly reflecting a change in conformation or overall shape of the IgG molecule due to binding with the gold particle, which could conceivably affect access of the gold-labelled IgG to the elastin. We found that under the conditions we used to detect HNE in polymorphonuclear neutrophil granules, our control using no primary antibody with goat serum blocking agent gave negative results.

As Damiano correctly points out, there is non-specific binding of immunogold when formalin is used to fix the sections. For this reason we used another method of fixation which resulted in virtually zero background levels, and correction for background labelling was not necessary. Although, as Damiano says, there was no label observed over elastic tissue for either the control or emphysematous lung tissue, this could not be due to the blocking of specific antigenic sites by normal goat serum as he suggests, as polymorphonuclear granules in the same sections were positively stained (we did not assess sections if polymorphonuclear granules were absent, as these were used as the internal control). We did, in fact, use a range of dilutions of primary and secondary antisera and blocking agents, from 1/10 to 1/10,000. The dilutions used were those in which there was the largest signal to noise ratio which, by coincidence, was the same dilution as that used by Damiano et al. We do think that HNE binds to elastin to degrade the substrate, and there are data to suggest that HNE binds tightly to elastin. There are, to our knowledge, however, no data to suggest that the reaction is irreversible. It is unusual in nature for an enzyme, which is essentially a catalyst, to bind irreversibly to its natural substrate. In doing so, it would presumably not be able to proteolyse its substrate or to have access to other substrate molecules, a self-defeating process which is more likely to result in less substrate degradation. We therefore believe that the enzyme degrades the elastin and is released at the same time as the molecule is cleaved. The exact kinetics that occur in vivo, particularly in the presence of endogenous inhibitors of the enzyme, are not understood. In our in vitro study, however, the elastin-enzyme preparation was washed in buffer to remove unbound enzyme and may have removed residually "bound" HNE; this explains our observation of the absence of specific binding of HNE to elastin in vitro.

In summary, we have developed an immunogold labelling technique that will specifically identify HNE when we know it to be present; thus we found it in polymorphonuclear neutrophil granules in human lung and in rat lung after intratracheal instillation. The fact that we could not find HNE in association with elastic tissue in the same sections of emphysematous human lung leads us to believe that it does not bind irreversibly to elastic tissue. We think that the principal methodological difference between the two studies[1] is our use of goat serum as a blocking agent. We wonder if Damiano et al used a blocking agent on their tissue sections which was homologous to the second antibody. This is a well established technique.[4]

References


Tributyrin hydrolysis for identifying Branhamella catarrhalis

The Tributyrin test (LAB M) as described by Richards' has been in use in this laboratory for over two years. Initially it was used as a final confirmatory test along with a DNase test (after screening by colonial appearance/Gram film/positive oxidase test/negative sugar utilisation tests (Oxoid, Neisseria identification discs), and it was always found to correlate directly with a positive DNase test. It is a rapid and very convenient test and was never found to be positive with any other species erroneously "picked" as potential Branhamella catarrhalis. Accordingly, the Tributyrin test was then used as a sole confirmatory test (after screening by appropriate colonial appearance/Gram film/positive oxidase test) for routine respiratory and conjunctival isolates. Systemic isolates were identified using all the tests in combination.

Ahmad et al made no reference to the Tributyrin test in the identification of B catarrhalis and advocated the use of the Superoxol test. He found that the National Collection of Type Culture strains of B catarrhalis and the strains designated Branhamella species identified as B catarrhalis (both DNase and Superoxol positive). Strain code number NC03623, however, is atypical on several counts; it produces smaller colonies and is both Tributyrin and DNase negative and produces a "slower" Superoxol reaction. The negative DNase test has been confirmed by the NCTC and this strain is no longer regarded as a typical B catarrhalis. The fact that the Tributyrin test readily picked up this anomalous strain whereas the Superoxol test did not would suggest that it is a more discriminating test for the identification of B catarrhalis.

PT MANNION

Brighton Public Health Laboratory, Royal Sussex County Hospital, Brighton, East Sussex.

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P T Mannion

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