**Effect of aerosol challenge with sensitising antigen on the permeability of the surface of the rat trachea in life**

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**ABSTRACT** A rat model of immediate pulmonary hypersensitivity was used to investigate the permeability changes in the tracheal epithelium produced by aerosol challenge with antigen. The rats were sensitised by the intraperitoneal injection of antigen (dinitrophenyl (DNP) ovalbumin). Sensitised and control animals were then challenged for 60 minutes with an aerosol of the same antigen, which also contained the electron dense pore marker lanthanum. Histological examination and x ray probe microanalysis showed a greater intercellular concentration of lanthanum in the tracheal epithelium in sensitised than in control animals. The results show that in sensitised rats increased intercellular penetrance of antigen can occur after antigen challenge.

**Introduction**

Antigen challenge of sensitised monkeys leads to increased concentrations of inhaled radiolabelled albumin in the blood, suggesting that sensitisation is associated with increased airways permeability to antigen. Allergen challenge has also been shown to increase the blood concentration and bronchoconstrictor effect of aerosolised radiolabelled histamine.

The sensitised rat is a useful model for investigation of the histological effects of immediate pulmonary hypersensitivity. We previously investigated the route of penetration of inhaled antigen into the pulmonary tissue by using a radioactive aerosol of antigen (dinitrophenyl ovalbumin—DNP ovalbumin). After challenge with tritiated DNP ovalbumin sensitised rats had more radioactive antigen in pulmonary tissue than unsensitised rats and the spaces between ciliated tracheal cells were wider. The use of a pore marker, lanthanum, confirmed increased penetration along the intercellular boundaries when the aerosol was combined with a fixative. The possibility that lanthanum had penetrated from the submucosa was not excluded in that experiment. It is also possible that mucosal permeability may not be increased in life and that fixation itself produces the apparent increase in permeability. We have therefore examined whether aerosol challenge of sensitised animals with antigen causes an increase in permeability of the respiratory epithelium lining in life.

**Methods**

**PROCEDURES** Inbred Chester Beatty rats maintained in the University of Bristol Medical School animal house were used. Males weighing about 200 g were injected intraperitoneally with saline (group A) or 10 μg DNP ovalbumin (group B) 21 days before challenge. A booster intraperitoneal injection of 1 μg of the same antigen was given three days before challenge.

**AEROSOL CHALLENGE** All animals were anaesthetised with 10 mg/kg intraperitoneal sodium pentobarbitol (Sagatal) and while breathing spontaneously were placed in a constant volume body plethysmograph. All were exposed for one hour to 20 mg/ml DNP ovalbumin containing 3% lanthanum nitrate, presented as an aerosol produced from a Bennett twin nebuliser. The nebulise was changed every 15 minutes to maintain its (measured) osmolality around 280 mosmol/kg, which is isosmotic with rat plasma.

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RESPIRATORY RESPONSE

Respiratory reactivity to aerosol challenge was measured as the percentage of expirations showing "expiratory notching," measured over more than 20 breaths. Expiratory notching being defined as a brief interruption of expiratory flow. We have previously shown it to be a good index of the pulmonary hypersensitivity reaction in the rat. The person assessing pulmonary reactivity and lanthanum penetration (SM) was unaware of which animals were sensitised.

TISSUE PREPARATION AND ELECTRON MICROSCOPY

Rats were killed immediately after challenge. Tracheal tissue was excised and fixed by immersion in 3% glutaraldehyde in phosphate buffered saline (pH 7.3) at 4°C for five hours. It was rinsed overnight in phosphate buffered saline at 4°C and then postfixed in 1% osmium tetroxide in phosphate buffered saline at room temperature for two hours. The tissues were dehydrated in graded ethanol solutions and embedded in epoxy resin (Araldite). Pale gold sections (about 600 Å thick) were cut and mounted on copper grids. The grids were placed in a Hexalord graphite spectrometer holder with a Faraday cage and viewed unstained in a JEOL 200 CX TEM/SCAN analytical electron microscope fitted with a KeVex 500 A/6000 x ray energy spectrometer. Transmission electron micrographs of sections were prepared.

The lanthanum content of 260 μm² areas of epithelium at sites below the level of the tight junctions was measured by means of x ray microanalysis in the scanning transmission mode. X ray analysis was standardised for a live time of 100 seconds and counts are expressed as mean counts/100 seconds. Ten sections were analysed for each animal and the results expressed as the ratio (P-B)/W (P denotes peak lanthanum counts, B background x ray counts, and W white radiation). White radiation is a factor used to cancel out any non-specific x rays and variations due to tissue thickness.

ANALYSIS

The data were analysed by means of Student's t test and results are presented as means and standard errors.

Results

Lanthanum was detected in the lumen, microvilli, and intercellular spaces in the sensitised animals (group B) after challenge with DNP₁₀ ovalbumin (fig 1). Lanthanum concentration was greatest at the airway surface and decreased progressively towards the connective tissue. In contrast, the unsensitised rats show negligible lanthanum in the lumen or within the tissue (fig 2):

only careful search shows some luminal lanthanum in control tissues (fig 2, insert). X ray probe microanalysis showed more lanthanum in the epithelium of group B than group A rats (mean 43 (SEM 6.5) v 12 (1.6); p < 0.01).

The respiratory response to challenge was greater in the sensitised rats than in the unsensitised rats (p < 0.05). The severity of respiratory response and quantity of lanthanum below the epithelial surface in the DNP₁₀ ovalbumin sensitised rats were not, however, significantly correlated (Spearman coefficient = 0.73; 0.05 < p < 0.1).

Discussion

The combination of an aerosol of allergen with electron dense lanthanum has permitted lanthanum to be used as a marker in electron microscope studies of tracheal permeability. Lanthanum was found to have penetrated the intercellular boundaries of the res-
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piratory epithelium in sensitised rats whereas there was very little penetration in unsensitised rats. The results suggest that there was increased permeability from the luminal surface of the trachea in life.

There was an apparent increase in lanthanum in the tracheal lumen and epithelial surfaces of sensitised animals. This might imply that the increase in intraepithelial lanthanum may be secondary to increased surface lanthanum. As the experimenter (SM) did not know whether an individual rat was sensitised, this cannot be due to experimenter bias. Equal quantities of lanthanum should have been deposited in the two groups of rats as there was equal exposure to the aerosol. Bronchial narrowing in the sensitised animals could have caused increased deposition of aerosol in the central airways, but this is unlikely as bronchoconstriction did not occur or was minor in our previous studies. The increased epithelial content could have arisen from epithelial disruption similar to that known to occur in asthma and after antigen challenge in animals. This would expose more membranes that the lanthanum could stain or it might act to reduce the microciliary clearance of lanthanum. The increase in luminal lanthanum might be due to leaching out of intraepithelial lanthanum or allergen in the fixation process or it might indicate a difference in the quantity or character of tracheal secretions. An increase in radiolabelled luminal allergen was noted in previous experiments when sensitised subjects were challenged.

Our previous study showed that aerosol challenge in sensitised rats leads to increased postmortem penetration of lanthanum along the intercellular boundaries. In the present experiments the lanthanum was applied only in life and postmortem artefact is less likely to explain our findings. The previous results also make it unlikely that the increased lanthanum deep to the tight junctions was merely a result of increased lanthanum at the luminal surface. The present results confirm that after antigen challenge more radioactive antigen is found in trachea and lung tissues from sensitised than from unsensitised rats.

Our previous work showed that sensitisation of rats produces an IgE mediated airway response that can be detected by volume plethysmography and blocked by pretreatment with cromoglycate. We have found the measurement of expiratory notching to be a sensitive indicator of bronchoconstriction in the rat. The sensitised group showed a greater increase in expiratory notching on challenge, though the magnitude of this response did not correlate with lanthanum pene-
tetration, possibly because of the small numbers of rats (six) examined. In a previous study lanthanum penetra-
tion in the fixed trachea showed a significant
relationship with the mechanical response.\(^7\) In the
latter system lanthanum is included in all fixing
solutions, so it may be measured more reproducibly
than in the present system, where it may be lost from
tissues during processing.

Although our results do not show directly that tight
inter epithelial junctions are open, this is a reasonable
extension from other work,\(^12\) which showed that
lanthanum penetration was closely related to the
patency of tight junction regions. Inflammatory events
in the airway may reduce the tightness of the inter-
cellular junctions,\(^13\) thereby facilitating the penetra-
tion of inhaled antigens or agonists to the submucosal
irritant receptors. A mucosal mechanism that allows
increased penetration has been suggested as the cause
of increased reactivity after cigarette smoke expos-
ure,\(^14\) ozone challenge,\(^15\) and viral infections.\(^16\)
Inflammatory reactions change airway permeability
in the guinea pig.\(^17\) Elwood et al\(^18\) failed to show any
increase in permeability to diethylene triamine penta-
acetate (DTPA) in asthmatic subjects, though they did
not challenge their subjects with antigen and so the
epithelium could have been in a resting state with
intra epithelial junctions closed. DTPA has been said
to be unsuitable as a marker of permeability as its
clearance after deposition in the airways is affected by
both the volume and the nature of the secretions,\(^19\)
both of which are altered in asthma.\(^20\)

The present work supports the hypothesis that
antigen challenge increases mucosal permeability. The
results suggest that this increase in permeability is due
to increased intercellular penetration. The increase in
permeability is most likely to be due to opening of the
tight junctions, though freeze fracture and membrane
potential studies following allergen challenge are
required to confirm this. Increased permeability could
progressively facilitate the penetration of agonists or
allergen or both to the deeper mucosal tissues and
accentuate the airway response.

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