The respiratory system in health and disease : Friday March 1 1996 / The Wellcome Centre for Medical Science.

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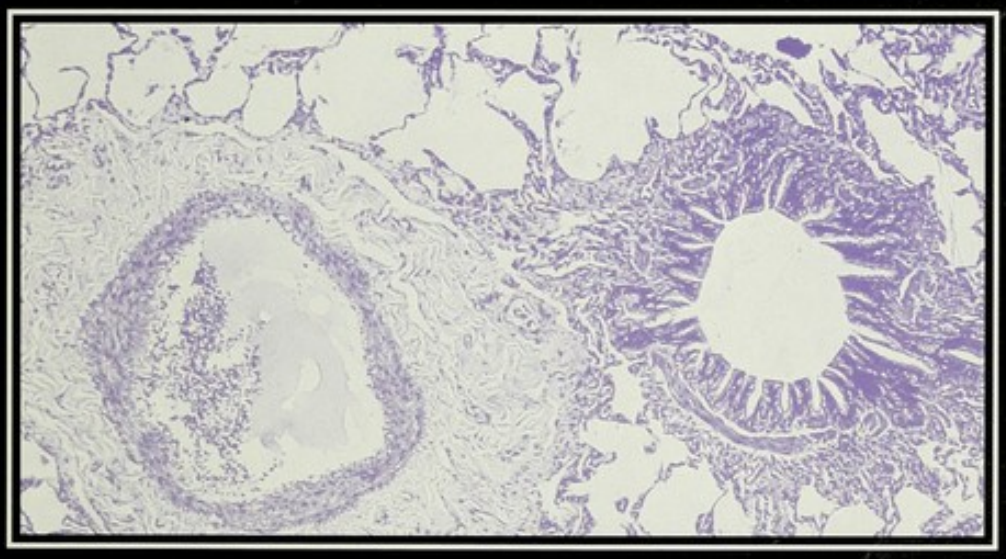
THE RESPIRATORY SYSTEM IN HEALTH AND DISEASE

Friday 1 March 1996



The Wellcome Building,
183 Euston Road, London NW1 2BE

THE RESPIRATORY SYSTEM IN HEALTH AND DISEASE

Friday 1 March 1995

The Wellcome Centre for Medical Science
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Acknowledgements

This is the fifth in a series of meetings for Trust-funded scientists that aims to gather together people working in different, but related areas. The meetings are also designed to encourage active participation of younger members of research groups. Previous, similar events include 'Diabetes' (March 1992), 'Red Blood Cells' (March 1993) and 'Bone, Cartilage and Extracellular Matrix' (March 1994) and 'Skeletal Muscle: Form and Function in Health and Disease' (March 1995).

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Friday 1 March 1996

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Professor Jonathan Lamb

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Professor Richard Olver

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Professor Jonathan Lamb

10.40 — G-protein regulation of alveolar sodium channels; why babies don't drown at birth
Dr Paul Kemp

11.00 — Cellular and molecular mechanisms of airway smooth muscle proliferation
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Speakers Abstracts

DISORDERED PHYSIOLOGY AND LUNG DISEASE: THE EARLY YEARS

Richard Olver
Dept of Child Health, Ninewells Hospital and Medical School, Dundee DD1 9SY

Abstract for Professor Olver will be circulated on the day of the meeting.

IMMUNOLOGICAL ASPECTS OF RESPIRATORY ALLERGY AND ASTHMA

Jonathan R Lamb
Infection and Immunity Section, Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB

The primary aim of this abstract is to provide a description of the immunopathology mediated by the inflammatory cell infiltrate that occurs in the late phase reaction (LPR), which in many instances, are characteristic of both allergic rhinitis and asthma. Furthermore, $CD4^+$ T cells are a major cellular component of LPRs and as such are of interest as targets for therapeutic intervention in allergic diseases.

Allergen exposure initiates the immediate symptoms of allergic rhinitis, which occur as the result of IgE dependent activation of mast cells and basophils. This immediate response is followed by the LPR, which is delayed in time and develops as a result of inflammatory cell infiltration. IgE mediated mechanisms may also contribute to allergen-induced episodic exacerbation's often observed in atopic asthma. However, their role in the pathogenesis of chronic asthma, in both atopic and non-atopic individuals, is unclear, although mast cell mediators such as histamine and LTC4 are important inducers of bronchospasms and mucus production. Mucosal inflammation is a central pathological feature of chronic asthma and is observed together with the loss of airway surface epithelium, thickening of the reticular basement membrane and smooth muscle hypertrophy that occurs in advanced disease.

T lymphocytes ($CD4^+$) and eosinophils ($EG2^+$) appear to dominate the inflammatory infiltrates. Following local allergen provocation $CD4^+$ 'memory' T cells expressing CD25 are found in bronchoalveolar lavage (BAL) and biopsies of dermal, nasal and bronchial origin. From the analysis of cytokine specific mRNAs by *in situ* hybridization and the cytokine response profiles of T cells cultured from peripheral blood and disease sites it has been established that the $CD4^+$ cells of the Th2 (IL-4, IL-5, and IL-13) and to a lesser degree of the Th0 functional phenotype are present. Selected cytokines play a central role in eosinophil recruitment with endothelial-activating cytokines (e.g. IL-1, TNFα and IL-4) inducing adhesions molecules on endothelium, eosinophil-activating cytokines (e.g. IL-3, IL-5 and/or GM-CSF) stimulating the differentiation, growth and survival of eosinophils and chemokines (e.g. RANTES, MIP-1α, MCP-3 and Ckb10) directly mediating eosinophil migration.

In both occupational and intrinsic asthma an increase in $EG2^+$ eosinophils and $CD25^+$ T cells is also observed, but in the letter while IL-5 is present only minimal levels of IL-4 are detectable. Airway hyperresponsiveness correlates with number of both eosinophils and T cells. Treatment of moderately servere asthma with corticosteroids reduces the eosinophilia and mRNA for Th2 type cytokines concomitant with a small increase in IFN-γ. A similar pattern of reciprocal regulation of Th2 and Th1 derived cytokines is also observed following the treatment of allergic inflammation by specific immunotherapy. The contribution of current forms of immunotherapy in the treatment of asthma appears to be limited. However, for the time being, $CD4^+$ T cells are likely to remain a major target for the development potential therapeutics in the treatment of allergic disease.

References

1 Gleich, G J and Kay, A B. (eds) *Eosinophils in Allergy and Inflammation.* Marcel Dekker, Inc., New York, 1993

2 Holgate, S T and Church M K. *Allergy.* Gower Medical Publishing, London, 1992.

3 Corrigan, C J and Kay, A B. 1992. *Immunol. Today,* **13**, 501–506.

4 Bochner, B S, Undem, B J and Lichtenstein, L M. 1994. *Ann. Rev. Immunol.,* **12**, 295–335.

5 O'Hehir, R E, Garman, R D, Greenstein, J L and Lamb, J R. 1991. *Ann. Rev Immunol.,* **9**, 67–95.

6 Robinson, D S, Hamid, Q A, Bentley, A, Sun, Ying, *et al.* 1993. *J. Allergy Clin. Immunol.,* **92**, 313–324.

7 Durham, S R, Sun Ying, Varney, V A, Jacobson, M R, *et al.* 1992. *J. Immunol.,* **148**, 2390–2394.

8 Varney, V A, Hamid, Q A, Gaga, M, Sun Ying et al. 1993. J. *Clin. Invest.* **92**, 644–651.

9 Hetzel, C, Hoyne, G F, Kristensen, N M, Bourne, T, *et al.* 1995. Urban, R G and Chicz, R M. *MHC Molecules: expression, assembly and function.* RG Landis Co. 261–279.

10 Romagnani, S. 1994. *Ann. Rev. Immunol.* **12**, 227–257.

G-PROTEIN REGULATION OF ALVEOLAR SODIUM CHANNELS; WHY BABIES DON'T DROWN AT BIRTH

Gregor K Fyfe, Paul J Kemp, Somnath Mukhopadhyay and Richard E Olver
Department of Child Health, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY

Postnatally, vectorial lung epithelial ion transport prevents alveolar flooding, but in the fetus, alveolar flooding is part of a physiological process in which an actively secreted liquid provides a template around which the developing airspaces grow. Interference with this dynamic process results in lung malformation. Accumulated evidence from studies on intact lung indicate that alveolar fluid absorption is dependent upon activation of transepithelial Na^+ transport.[1] The functional switch from secretion to absorption is initiated at birth by a β_2 adrenoceptor-mediated rise in cAMP which activates amiloride-sensitive Na^+ conductance in the epithelial apical membrane.[2] Inadequate activation of this mechanism contributes significantly to neonatal respiratory distress.

To investigate the roles of the separate components involved in alveolar fluid homeostasis and its regulation at birth, we have developed preparations of isolated alveolar type II (ATII) cells and purified ATII apical membrane vesicles (AMV) of mature fetal guinea pig lungs.

The conclusion that the apical membrane of the ATII cell is the locus of the Na^+ absorptive step of fluid clearance is based on the observations that:

1 excised patches of ATII cells exhibit a low conductance, Na^+-selective, amiloride-sensitive channel;[3]
2 AMV possess an analogous conductive $^{22}Na^+$ flux which is blockable by amiloride and phenamil;[4]
3 incorporation of AMV into lipid bilayers results in functional reconstitution of the relevant channel.[5]

Using patch-clamp and flux measurements we have shown that ATII channels are up-regulated both by co-localized G proteins and arachidonic acid (AA). The modulation by AA is independent of its metabolism, can be mimicked by a range of structurally diverse fatty acids (FA) – both saturated and unsaturated – and is not attenuated by functional inactivation of G proteins suggesting a role for a direct FA/Na^+ channel interaction. However, the system is complicated by the observation that G protein activity is acutely sensitive to unsaturated FA which suggests that there are two overlapping FA-dependent pathways involved in modulation of fetal alveolar Na^+ transport. The G protein-dependent pathway is possibly the physiologically more important regulatory route since it shows specificity for unsaturated FA; it also represents a novel and additional level of control of signal transduction in the developing lung to that classically described merely in terms of receptor/G protein interactions and may be a negative feedback loop for the FA signal.

Interruption of specific G protein cycling, using toxin-dependent ribosylation, has revealed that unsaturated FA exert their effects via pertussis toxin (PTX)-insensitive G proteins. However, the regulation of Na^+ channels by exogenous GTP analogues has a significant component which is PTX-sensitive. Therefore, these two regulatory pathways (direct G protein versus FA/G protein) are effective via distinct G protein isoforms.

How these multilayered regulatory systems integrate to produce the functional switch at birth and subsequent fine tuning of alveolar fluid transport has major implications for understanding of lung fluid homeostasis in health and disease.

References

1 Olver, R E, Ramsden, C A, Strang, L B and Walters, L B. 1986. *J. Physiol.* **376**, 321–340.
2 Walters, D V, Ramsden, C A and Olver, R E. 1990. *J. Appl. Physiol.* **68**, 2054–259.
3 MacGregor, G G, Olver, R E and Kemp, P J. 1994. *Am. J. Physiol.* **267**, L1–L8.
4 Fyfe, G K, Kemp, P J, Cragoe, E J, and Olver, R E. 1994. *Biochim. Biophys. Acta* **1224**, 355–364.
5 Kemp, J, Fyfe, G K and Ashley, R H. 1995. *J. Physiol.* **487**, 195–196P.

CELLULAR AND MOLECULAR MECHANISMS OF AIRWAY SMOOTH MUSCLE PROLIFERATION

*Stuart J Hirst, Peter J Barnes and Charles HC Twort**
UMDS Department of Allergy and Respiratory Medicine, St Thomas' Hospital, London SE1 7EH
**National Heart and Lung Institute, Dovehouse Street, London SW3 6LY*

Resistance to laminar flow in the peripheral airways is related inversely to the fourth power of the luminal radius. Small changes in airway lumen diameter cause profound increases in airway resistance to airflow. Increased contraction of airway smooth muscle (ASM) was thought to account for this reduction in airflow, but it is now well established that in chronic severe asthma increases in airway wall thickening due to an increase in the smooth muscle mass can, even in the absence of smooth muscle contraction, account for the irreversibility to bronchodilators and the bronchial hyperresponsiveness characteristic of severe asthma. The pathophysiological nature and source of the mediators controlling airway wall remodelling is unknown, and until very recently has received little attention.[1]

We have developed a model preparation of human bronchial smooth muscle cells in culture to study the proliferative response.[2] The effect of recombinant PDGF isoforms (PDGF-AA, -BB and -AB) on mitogenesis was examined using the MTT reduction assay and [^{3}H]thymidine incorporation. We have also examined possible roles for protein kinase C (PKC) and protein tyrosine kinase (PTK) signalling pathways in PDGF-stimulated mitogenesis of human ASM cells.

Results were correlated with expression of PDGF receptor (PDGFR) α and β subunits in the absence and presence of fetal calf serum (FCS). When FCS was absent PDGF-AB and -BB were potent mitogens, while PDGF-AA was weakly mitogenic, evoking <20% of the maximum response induced by the B-chain isoforms. When FCS (2.5%) was present, all PDGF isoforms stimulated marked ASM proliferation with similar efficacy and potency. Cross-competition binding analysis in FCS-deprived cells revealed that ASM cells in culture express mainly PDGFRβ. Preincubation with PDGF-AA or PDGFRα neutralizing anti-serum abolished PDGF-AA binding and decreased total receptor number by ~15%. The ratio of PDGFR α:β subunits was ~1:8, supported by intense immunofluorescence staining for PDGFRβ and weak staining for PDGFRα.[3] In parallel studies uptake of [^{3}H]thymidine stimulated by PDGF-AA, but not PDGF-AB or -BB, was inhibited by PDGFRa immobilization. Western immunoblots confirmed expression of mature PDGFRα and β subunits. FCS did not cause any detectable increase in PDGFRα expression or in PDGF-AA binding. The involvement of PKC- and PTK-dependent signalling events was examined using pharmacological inhibitors. The relative selectivity of each protein kinase inhibitor for its intended target enzyme was determined. Ro31-8220 (0.001–10μM; PKC inhibitor), but not ST638 (0.001–100μM; PTK inhibitor), produced concentration- and ATP-dependent inhibition of partially purified Ca^{2+}-dependent PKC isolated from ASM cells. In contrast ST638, but not Ro31–8220, abolished the rise in ASM cell phosphotyrosine content. Ro31-8220 (0.001–3μM) and ST638 (0.1–100μM) inhibited PDGF isoform-stimulated DNA synthesis and proliferation (n=4–8), and were equipotent with respect to each PDGF isoform. The inhibition produced by Ro31-8220 and ST638 in combination, against any PDGF isoform, was no more than additive.

Our data support a role for PDGFRβ mediating ASM mitogenesis during FCS-free conditions, but in the presence of FCS both PDGFRα and β subunits are linked to mitogenesis. The enhanced mitogenicity of PDGF-AA in the presence of FCS was independent of any detectable up-regulation

of PDGFRα, suggesting that the inability of PDGF-AA to promote mitogenesis in the absence of FCS is not simply due to relative numbers of PDGFRα and PDGFRβ. Our results are also consistent with the involvement of PTK and PKC in PDGF isoform-stimulated ASM mitogenesis.

References

1 Hirst, S J and Twort, C H C, 1992. *Clin. Exp. Allergy*, **22**, 907–915.
2 Hirst, S J, Barnes, P J, and Twort, C H C. 1992. *Am. J. Resp. Cell Mol. Biol.*, 7, 574–581.
3 Hirst, S J, Barnes, P J, and Twort, C H C. 1995. *Am. J. Physiol.*, (in press).

EOTAXIN: THE DISCOVERY OF AN EOSINOPHIL CHEMOATTRACTANT CHEMOKINE WITH POTENTIAL IMPORTANCE IN ASTHMA

P D Collins, P J Jose, D A Griffiths-Johnson, A A Humbles, S Marleau, S Larkin, S Nourshargh, D Conroy and T J Williams
Applied Pharmacology, National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, Dovehouse Street, London SW3 6LY

Eosinophil recruitment is a prominent feature of allergic reactions and is implicated in the tissue damage and bronchial hyperresponsiveness associated with asthma. The accumulation of these leukocytes from microvessels is regulated by the local production of chemoattractant mediators. We have recently identified a potent and selective eosinophil chemoattractant, 'Eotaxin', generated in the bronchoalveolar lavage fluid (BALF) of allergen-challenged/sensitized guinea pigs.[1] Eotaxin is a novel member of the C–C branch of the chemokine family of chemoattractant cytokines which also includes monocyte chemotactic protein (MCP) and RANTES.

Eotaxin is very potent *in vivo*, causing substantial eosinophil accumulation in guinea pig skin at doses of 1–2 pmoles[1] and eosinophil influx in the lung after administration of Eotaxin by aerosol.[2] Further, marked synergism with interleukin-5 has been demonstrated.[3] Intravital microscopy of the guinea pig mesentery shows that topical Eotaxin administration induces intravascular eosinophil rolling and adherence within minutes, followed by migration through the microvascular endothelium of post-capillary venules. *In vitro*, Eotaxin activates both guinea pig and human eosinophils inducing calcium mobilization and cell–cell aggregation.[1,2] In contrast, the human eosinophil-active chemokine RANTES, while competing with Eotaxin for binding sites on guinea pig cells, does not activate them.[4] Hence, we have demonstrated that human RANTES acts as receptor antagonist for Eotaxin in the guinea pig.[4]

There is a low basal expression of Eotaxin mRNA and protein in the lung which is considerably enhanced following allergen challenge.[5] Using a specific radioimmunoassay, peak levels of Eotaxin in BALF were found 6 hours after challenge and all the chemoattractant activity in 6h BALF concentrates was neutralized by anti-Eotaxin antibodies.

The demonstration that a selective eosinophil chemoattractant, Eotaxin, is generated in a model of allergic airways inflammation (murine and human Eotaxin have been identified) offers the opportunity to develop a potential therapy that targets Eotaxin release or action. We have provided evidence that one such therapeutic intervention might be based around antagonism of the Eotaxin receptor.

References

1 Jose, P J, Griffiths Johnson, D A, Collins, P D, Walsh, D T, Moqbel, R, Totty, N F, Truong, O, Hsuan, J J and Williams T J. 1994. Eotaxin: A potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.*, **179**, 881.

2 Griffiths Johnson, D A, Collins, P D, Rossi, A G, Jose, P J, and Williams, T J. 1993. The chemokine, Eotaxin, activates guinea pig eosinophils in vitro, and causes their accumulation into the lung *in vivo. Biochem. Biophys. Res. Commun.*, **197**, 1167.

3 Collins, P D, Marleau, S, Griffiths Johnson, D A, Jose, P J, and Williams, T J. 1995. Cooperation between interleukin-5 and the chemokine Eotaxin to induce eosinophil accumulation *in vivo. J. Exp. Med.*, **182**, 1169.

4 Marleau, S, Griffiths-Johnson, D A, Collins, P D, Bakhle, Y S, Schall, T J, Williams, T J, and Jose, P J. 1995. Human RANTES acts as a receptor antagonist for guinea pig eotaxin *in vitro* and *in vivo*. *J. Exp. Med.* (submitted).

5 Jose, P J, Adcock, I M, Griffiths Johnson, D A, Berkman, N T, Wells, N C, Williams, T J, and Power, C A. 1994. Eotaxin: Cloning of an eosinophil chemoattractant cytokine and increased mRNA expression in allergen challenged guinea pig lungs. *Biochem. Biophys. Res. Commun.*, **205**, 788.

PUTATIVE ROLE OF CADMIUM IN PULMONARY EMPHYSEMA

Rachel C Chambers
Centre for Cardiopulmonary Biochemistry and Respiratory Medicine, The Rayne Institute, University College London Medical School, 5 University Street, London WC1E 6JJ

Chronic inhalation of cadmium (Cd^{2+}) fumes is associated with the development of emphysema, a disease characterized by extensive disruption of lung connective tissue. Cd^{2+} is also an important contaminant of tobacco and may play a role in smoking-induced emphysema. The mechanism by which Cd^{2+} induces these changes is poorly understood. We hypothesized that, part of the action of Cd^{2+} in the lung is via direct effects on lung connective tissue repair processes. To begin to address this hypothesis, we examined the effect of non-cytotoxic doses of cadmium chloride ($CdCl_2$) on connective tissue protein synthesis and proliferation by cultured fibroblasts.

Cd^{2+} inhibited procollagen[1,2] and proteoglycan[3] synthesis in a dose-dependent manner. For fetal rat fibroblasts, maximal effects were observed at 10μM-$CdCl_2$, with procollagen production values, relative to media controls, reduced by 81±2% (mean ± SEM; n=6; p<0.01). In contrast, non-collagen protein synthesis by these cells was increased at all doses of $CdCl_2$ examined. Non-collagen protein synthesis was unaffected by Cd^{2+} in human fetal lung fibroblasts. However, procollagen production was maximally inhibited by 83±2% (p<0.01) at 40μM-$CdCl_2$. In human cells, proteoglycan synthesis was inhibited from 30μM onwards, with values significantly reduced by 36±4% and 42±6% (both p<0.01) for proteins secreted into the culture media and for proteins associated with the cell layer, respectively. Preliminary results obtained suggest that Cd^{2+} is exerting these effects pre-transcriptionally since mRNA steady state levels for procollagen, decorin and versican were reduced by between 40% and 60% in the presence of 30μM-$CdCl_2$. The effect of Cd^{2+} on human fibroblast procollagen metabolism, in response to transforming growth factor-β1 ($TGF\beta_1$), was also examined in order to determine whether Cd^{2+} inhibits fibroblast responses to mediators known to play an important role in upregulating connective tissue synthesis and deposition during tissue repair. Cd^{2+}, at doses which did not affect basal rates of procollagen production, attenuated the stimulation induced in response to TGFβ1 by 64% (p<0.01).[4]

Cd^{2+} was also a potent inhibitor of fibroblast proliferation in response to serum-derived mediators. Replication for rat fibroblasts in response to 2% serum was reduced by 17±4%, 72±2% and 86±4% (all p<0.01) at 1μM, 5μM and 10μM-$CdCl_2$ over a 24 hour incubation period. In human fibroblasts, proliferation was significantly reduced from 10μM-$CdCl_2$ onwards, with values reduced by 13±3% (p<0.05), 38±4% and 41±5% (both p<0.01) at 10μM, 15μM and 20μM-$CdCl_2$, respectively.[1]

These data support the hypothesis that Cd^{2+}, at doses likely to be attained in the lung, inhibits key processes of connective tissue repair. These effects of Cd^{2+}, in the face of ongoing oxidant and phagocyte-mediated tissue damage, are likely to play an important role in the pathogenesis of emphysema in humans, chronically exposed to Cd^{2+} fumes occupationally or in cigarette smoke. Current research is focused on examining the mechanism by which Cd^{2+} exerts these effects on fibroblasts and on evaluating the importance of these effects *in vivo*.

References

1 Chambers, R C, McAnulty, R J, Shock, A, Campa, J S, Newman Taylor, A J, and Laurent, G J. 1994. *Am. J. Physiol.*, **267**, L300–L308.

2 Chambers, R C, and Laurent, G J. 1995. In: Correlations between *in vitro* and *in vivo Investigations in Inhalation Toxicology, ILSI Press* (in press).

3 Chambers, R C, Westergren Thorsson, G, McAnulty, R J, Malmstrom, A, Newman Taylor, A J, and Laurent, G J. 1994. *Am. Rev. Respir. Dis.*, **149**, A629.

4 McAnulty, R J, Chambers, R C and Laurent, G J. 1994. *Am Rev Respir Dis.*, **149**, A89.

DEVELOPMENTAL CHANGES IN OXYGEN SENSITIVITY OF CATECHOLAMINE SECRETING CELLS

Mart H Mojet, Elliott Mills, Mark Hanson and Michael R Duchen
Dept of Physiology, University College London, Gower Street, London, WC1E 6BT

Adrenal chromaffin cells and the oxygen sensing Type I cells of the carotid body are ontogenetically closely related. As such, both secrete catecholamines in response to a decrease in oxygen partial pressure (PO_2). In addition, the sensitivity of each system changes within the first two weeks of life: where the sensitivity of the carotid body to hypoxia increases, the responsiveness of the adrenal medulla decreases. We have addressed the cellular processes underlying these responses and their modification during development.

Using isolated cells, we have employed microfluorimetric and fluorescence imaging techniques to examine changes in mitochondrial function and intracelular free calcium ($[Ca^{2+}]_i$), the amphotericin perforated patch clamp technique to study changes in membrane current or membrane potential, and amperometry at carbon fibre microelectrodes to study catecholamine secretion. Using the latter technique, the secretion of individual catecholamine containing vesicles was resolved. As some of these techniques can be applied simultaneously, we have also been able to assess quantitative and temporal relationships between these variables.

Systematic examination of the specific oxygen sensitivity of the rat adrenal chromaffin cells revealed that in newborn rats severe hypoxia induces a depolarisation of mitochondrial potential, an increase in NADH/NAD$^+$ ratio, a rise in $[Ca^{2+}]_i$ and an increase in catecholamine secretion. Thus, the secretion of catecholamines from the adrenal during neonatal asphyxia is exocytotic in nature and reflects an intrinsic response property of the chromaffin cells. An equivalent set of responses is seen to cyanide, a specific inhibitor of mitochondrial respiration. This correlation suggests that hypoxia is sensed in the mitochondria. The increase in ($[Ca^{2+}]_i$) stems from Ca^{2+} influx through plasma membrane channels, as it is blocked by D-600 and requires the presence of external Ca^{2+}. We are currently attempting to identify the mechanisms that couple inhibition of mitochondrial respiration to an increase in Ca^{2+} influx. In the neonatal period, asphyxia-induced catecholamine secretion plays a role in prolonging survival and is also thought to affect lung compliance, resorption of lung fluid and production of surfactant, processes critical in establishing neonatal respiratory competence. Indeed, in animals at 2–4 weeks of age, cyanide still causes the expected changes in mitochondrial potential and redox state, but the changes in ($[Ca^{2+}]_i$ and secretion are substantially reduced.

Currently we are also examining the response properties of Type I cells of the sheep carotid body, as the developmental changes in this species have been well defined. Changes in sensitivity occur after birth, in order to maintain chemoreflexes in the face of the large rise in arterial PO_2, postnatally. However, very little is known of the properties of Type I cells of the sheep. Preliminary studies show that the cells respond to depolarisation and inhibition of mitochondrial respiration with changes in ($[Ca^{2+}]_i$ similar to the changes obtained in the newborn rat adrenal chromaffin cells. We are currently attempting to correlate changes in mitochondrial potential, ($[Ca^{2+}]_i$) and secretion with hypoxia, to compare the O_2 sensitivities between foetal and two week old animals.

RESPIRATORY CONTROL DURING 48 HOURS OF HYPOXIA IN HUMANS

L S G E Howard, M J Poulin and P A Robbins
University Laboratory of Physiology, Parks Road, Oxford OX1 3PT

In an earlier study, we demonstrated that, in humans, ventilation (V_E) increased dramatically over 8 hours of isocapnic hypoxia when compared with poikilocapnic hypoxia.[1] This rise was thought to be due to a direct effect of hypoxia *per se*, which was masked in the poikilocapnic exposure by the concomitant respiratory alkalosis. This conclusion was based on the finding that the changes in the acute hypoxic ventilatory response (AHVR) at matched $PETCO_2$ were not significantly different between the esocapnic and poikilocapnic exposures.[2]

In order to examine the respiratory response over a time course during which ventilatory acclimatization to the hypoxia of altitude is known to take place, we decided to extend the period of hypoxic exposure to 48 hours. In addition, we observed the 48-hour period following hypoxic exposure, so-called deacclimatisation.

Four subjects were studied. Each was exposed to two different 48-hr protocols in a purpose-built chamber, in which the ambient CO_2 and O_2 partial pressures were adjusted at regular intervals by a computer-controlled system to generate the desired end-tidal gas composition in the subject (measured by nasal catheter). The two protocols employed were: (1) isocapnic hypoxia, IH, where for three subjects end-tidal PO_2 ($PETO_2$) was held at 60 mmHg and for one at 55 mmHg and where the end-tidal PCO_2($PETCO_2$) was held at the subject's resting value prior to experimentation; and (2) poikilocapnic hyoxia, PH, where $PETO_2$ was held at the values given above and $PETCO_2$ was left uncontrolled. During deacclimatisation, subjects were allowed home, but returned to the laboratory at set times for assessment. During the hypoxic exposure V_E was measured at two-hour intervals (except during sleep). AHVR was measured using a series of 6 square-wave changes in $PETO_2$, each with a period of two-min, alternating between 50 and 100 mmHg, which were imposed using a computer-controlled fast gas-mixing system. $PETCO_2$ was held at 2 mmHg above the subject's original resting value. A single-compartment model was used to estimate the component of ventilation sensitive to acoute changes in hypoxic stimulation, Gp (l/min/%), and the component insensitive to such changes, Vc (l/min). Eupnoeic ventilation was determined prior to each test of AHVR.

During IH, VE rose from 11077+1.10 l/min (mean+S.E.) to 29.08+3.26 l/min, and during PH, V_E rose from 9.88+0.68 to 13.3+1.22 l/min. The difference between the two protocols was significant ($p<0.001$, ANOVA). Eupnoeic ventilation (V_E at a $PETO_2$ of 100 mmHg with no added CO_2, as reflected by the fall in $PETCO_2$ rose significantly during both IH ($p<0.01$; paired t-test) and PH ($p<0.025$). During deacclimatisation, eupnoeic $PETCO_2$ increased following both types of exposure. There was no significant difference between the two protocols (ANOVA). In the tests of AHVR, Gp and Vc rose during IH and PH and fell during acclimatization. Again, there were no significant differences between the two protocols (ANOVA). In conclusion, it appears that the acid-base status of the hypoxic exposure plays little role in the respiratory changes over 48 hours of hypoxia and that hypoxia per se is the principal factor in the early stages of ventilatory acclimatisation to altitude.

This study was approved by the Central Oxford Research Ethics Committee.

References

1 Howard LSGE and Robbins PA 1995a. *J.Appl.Physiol.*, **78**, 1092–1097
2 Howard LSGE and Robbins PA 1995b. *J.Appl.Physiol.*, **78**, 1098–1107

HIGHER CENTRE CONTROL OF BREATHING IN MAN; EVIDENCE FROM IMAGING THE BRAIN

Douglas Corfield, Abe Guz, Lewis Adams and Kevin Murphy
Department of Medicine, Charing Cross Hospital, Fulham Palace Road, London, W6 8RF

The automatic generation of a respiratory rhythm within ponto-medullary structures and its projection via the bulbo-spinal tract has been extensively investigated in anaesthetised animals. The same spinal motoneurones can be activated from higher centres in the central nervous system allowing modulation of breathing for volitional and behavioural purposes. In man, such non-automatic control of breathing is responsible for taking or holding a breath, the production of speech and the emotional and arousal effects on breathing. This study attempts to define the brain areas involved in higher centre breathing control in normal man and whether such areas are involved in the ventilatory response to exercise and/or chemical stimulation with CO_2. We have used Positron Emission Tomography (PET) with I.V.$H_2{}^{15}0$ to demonstrate areas of increased local blood flow and hence neural activity. We have minimised 'noise' and controlled partly for afferent feedback from the chest by contrasting the results during the experimental state with a 'passive' control state using intermittent positive pressure ventilation. In each study data has been pooled from all the subjects. Our results show: 1) Voluntary inspiration (1.5–21) with maintained normocapnia is associated with focal activation bilaterally in the supero-lateral 1° motor cortex (MI);[1] these areas are similar to those which when stimulated transcranially with a magnetic pulse activate the contralateral diaphragm.[2] The scans also show activations in the supplementary motor cortex (SMA) and the lateral premotor cortex (PMA) bilaterally; these areas are known to be concerned with planning and organization of movement. 2) Voluntary expiration (1.5–21) against an expiratory threshold load of 10cm H_20 is associated with the same activations as for inspiration with the addition of large areas of activation in the ventrolateral MI;[3] such areas when stimulated directly during neurosurgery have given rise to vocalisation i.e. active expiration with laryngeal activity. 3) During leg exercise adequate to increase 02 uptake 2.5 fold with steady state ventilation raised to 23.0±2.9 $1.min^{-1}$, and $PaCO_2$ of 41.4±1.3 mmHg, the supero-lateral MI has been activated bilaterally in areas with coordinates similar to those found with volitional inspiration.[4] These activations together with those in SMA and PMA bilaterally occur in addition to the supero-medial MI activations of the motor-cortical leg areas. Post-exercise, while ventilation is still raised but decreasing, the same activations are present but without the MI leg areas. These results during and after leg exercise provide evidence for the anatomical/physiological basis of 'feed-forward' mechanisms in exercise-related hyperpnoea. 4) During CO_2-stimulated breathing (with breathlessnness) adequate to raise $PETCO_2$ to 50.3±1.7 mmHg, and ventilation to 27.4±2.1 $1.min^{-1}$, activation has been found in the brainstem as well as in the entire limbic system5. No activation was found in MI. These activations are likely to be of significance in the sensory and/or motor respiratory responses in hypercapnia. The control of airflow while speaking is currently being studied with PET. We also plan to scan cortex and medulla together with volitional/behavioural breathing acts to clarify how higher centre breathing control interacts with the automatic brainstem control system.

References

1 Colebatch J G, Adams L, Murphy K, Martin A J, Lammerstsma, A A *et al.* 1991. *J Physiol.*, **443**, 91–103

2 Maskill D, Murphy K, Mier A, Owen M and Guz A 1991. *J Physiol.*, **443**, 105–121

3 Ramsay S C, Adams L, Murphy K, Corfield D R, Grooton K S, *et al.* 1993. *J Physiol.*, **461**, 85–101
4 Fink G R, Adams L, Watson J D G, Innes J A, Wuyam B, *et al.* 1996. *J Physiol.*, - in press
5 Corfield D R, Fink G R, Ramsay S C, Murphy K, Harty H P *et al.* 1995. *J Physiol.*, **488,** (1), 77–84

CYTOLOGY OF BRONCHOALVEOLAR LAVAGE FLUID AND TRACHEAL RESPIRATORY SECRETIONS IN CLINICAL PULMONARY DISEASE IN HORSES

P M Dixon, B C McGorum and D I Railton
Dept of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, Edinburgh

Bronchoalveolar lavage fluid (BALF) and tracheal respiratory secretions (RS) were collected from 300 horses, 270 with pulmonary disease (mainly chronic, >2 months duration) and 30 controls. Cytology of these samples showed higher proportions of neutrophils in tracheal RS than in BALF in both diseased and control horses, e.g. 148 cases of chronic obstructive pulmonary disease (COPD) had median neutrophil proportions of 90% (range, 14–100) in RS and 25.7% (5.5–98) in BALF, 45 cases of infectious pulmonary disease had 45% (0–100) neutrophils in RS and 4% (0–42) in BALF, while the controls had 10% (0–68) neutrophils in RS and 2% (0–5) in BALF. Although RS neutrophil proportions were 4–10 times higher than BALF levels in control and disease groups, a highly significant ($p<0.001$) correlation was present between these two variables between all 300 horses.

Eosinophils were rarely detected in BALF or RS (median 0% eosinophils in both control and COPD groups) and only the lungworm infection group (7 cases) had raised BALF (20.5%, 6–58) and RS (57%, 0–98) eosinophil proportions.

The BALF neutrophil proportions (mean 2%) of the control horses in this study are lower than previously recorded equine values and are similar to human and canine control values. These results indicate that BALF cytology is of great diagnostic value in the investigation of clinical pulmonary disease in horses and that the presence of a chronic BALF neutrophilia (>5% neutrophils) is very indicative of COPD. However, the overlap in RS neutrophil ratios between control and diseased horses limits the diagnostic value of RS cytology in the clinical situation.

GENERATION AND CHARACTERIZATION OF A ΔF508 CYSTIC FIBROSIS MOUSE MODEL

Catharine A Goddard
Wellcome/CRC Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR

Cystic fibrosis (CF) is a fatal autosomal recessive genetic disease affecting about one in 2000 Caucasians. The disorder is characterized by defective transepithelial Cl^- ion transport in several organs including the airways, intestine, pancreas and sweat ducts. The major cause of morbidity and mortality results from hyper-accumulation of mucus in the airways which promotes recurrent microbial infections leading to fatal lung destruction. The gene responsible for CF, the cystic fibrosis transmembrane conductance regulator (CFTR), encodes a cAMP-regulated Cl^- channel spanning the apical membrane of epithelial cells. CF is caused by a variety of mutations in the CFTR gene. The most common mutation, accounting for approximately 70% of all mutant alleles, is a deletion of three base pairs which removes the phenylalanine at position 508 (ΔF508) from the first nucleotide binding domain of the protein. The ΔF508 mutation causes the incorrect processing of the protein such that it is retained in the endoplasmic reticulum (ER)[1] and subsequently degraded. Experiments have shown that either culturing cells expressing the ΔF508 protein below 33°C or causing over expression of the ΔF508 protein leads to the protein exiting the ER and inserting into the apical membrane where it forms a functional Cl^- ion channel.[2,3] Although the transgenic CF null mice have been useful for testing potential gene therapy stratagies[4–6] a mouse model for the ΔF508 mutation would allow the evaluation of pharmacological agents which could be used to overcome the trafficking defect. Here I present the evidence that mice carrying the ΔF508 mutation have been created and represent an accurate model for studying the ΔF508 processing defect. A replacement type targeting construct was used to introduce the ΔF508 mutation into exon 10 of the mouse *cftr* gene in embryonic stem (ES) cells. Correctly targeted ES cells were used to generate mice carrying the ΔF508 mutation. Homozygous mutant mice show pathological and electrophysiological changes consistent with a cystic fibrosis phenotype. $\Delta F508^{-/-}$ mice die from peritonitis caused by intestinal blockages and show deficiencies in cAMP activated Cl^- transport in both the airways and intestine. Quantitative RT-PCR analysis shows that the ΔF508 mice produce ΔF508 transcripts and SPQ fluorescence imaging shows that the ΔF508 mice show the same trafficking defect as shown for human ΔF508. Cells were isolated from the tracheas of either wild type, $\Delta F508^{-/-}$, or CF null mice and incubated at either 37°C or 27°C. The CF null mice showed no cAMP stimulated Cl^- conductance at either temperature as measured by the rate of change of SPQ fluorescence. However the ΔF508 mice showed cAMP stimulated Cl^- conductance at 27°C but not 37°C. This is consistent with the known ability of the protein to insert into the apical membrane at this temperature. Thus the ΔF508 mice provide a valid model for testing strategies for overcoming the trafficking defect associated with the ΔF508 mutation.

References

1 Cheng, S H, Gregory, R J, Marshall, J, Paul, S, Souza, D W, White, G A, O'Riordan, C R and Smith, A. 1990 *Cell*, **63**, 827–834

2 Denning, G M, Anderson, M P, Amara, J F, Marshall, J, Smith, A S, Welsh, M J. 1992, *Nature*, **358**, 761–764

3 Li, C M, Ramjeesingh, M, Reyes, E, Jensen, T, Chang, X-B, Rommens, J M and Bear, C E. 1993, *Nature Genetics*, **3**, 311–316

4 Grubb, B R, Pickles, R J, Ye, H, Yankaskas, J R, Vick, R N, Englehart, J F, Wilson, J M, Johnson, L G and Boucher, R C. 1994, *Nature*, **371**, 802–806
5 Alton, E W F W, *et al.* 1993, *Nat. Genetics*, **5**, 135–142
6 Hyde, S C, Gill, D R, Higgins, C F, Trezise, A O, MacVinish, L J, Cuthbert, A W, Ratcliff, R, Evans, M J and Colledge, W H. 1993, *Nature*, **362**, 250–255

GENE THERAPY FOR CYSTIC FIBROSIS

Eric Walter Frederick Wolfgang Alton and Myra Stern
Ion Transport Unit, National Heart and Lung Institute, Emmanuel Kaye Building, Manresa Road, London SW3 6LR

Since the cloning of the cystic fibrosis gene in 1989, gene therapy for this disease has become a possibility. Over the last few years our group has been involved in assessing the use of cationic liposome-mediated *CFTR* gene transfer in laboratory studies *in vitro*, in animal models of cystic fibrosis and most recently in the first clinical trial of liposome-mediated gene therapy for this disease. In preparation for studies in animal models and in patients, we developed a number of assay systems to measure the electrical abnormalities consequent upon dysfunction of the cystic fibrosis gene. Using these assays, we validated the use of a newly generated cystic fibrosis mouse to mimic the disease in man.[1] We then nebulized a normal copy of the human cystic fibrosis gene into this mouse model to assess whether it was possible to correct the basic biochemical defect. The data demonstrated an approximately 50% correction of the chloride transport defect towards wild type following gene transfer.[2] Following these studies, we went on to complete the first ever trial of cationic liposome-mediated gene therapy in cystic fibrosis patients. Fifteen subjects were assessed in a double blind placebo controlled study in which the human cystic fibrosis gene complexed with a cationic liposome was applied to the nasal epithelium. No safety problems were encountered and approximately 20% correction of the basic chloride defect towards normal levels was demonstrable.[3] One of the difficulties emerging from these studies relates to the minimum level of wild type CFTR messenger RNA required in each cell to achieve function correction. We have recently completed a study in which functional analysis of the various CF mouse models available has allowed us to construct a graph of the relationship between CFTR messenger RNA and function correction. The data suggest that very low levels of wild type messenger RNA (5% to 10% per cell) are sufficient to achieve marked correction of the basic functional abnormality.[4] We are now preparing a study of liposome-mediated CFTR gene transfer into the lungs of CF patients to assess the possibility of achieving functional correction at this site. Because liposome-mediated gene transfer to the lower airways has not previously been studied, new assays have been developed in preparation. These include the use of fluorescent dyes to assess chloride transport in individual cells obtained from the airway epithelium by a brushing technique.[5] In addition, we have recently demonstrated the validity of using bacterial adherence, which is increased in cystic fibrosis patients, as a further assay to monitor the efficacy of gene therapy. This provides the first clinical assay system developed to assess CFTR function. Finally, preparatory studies of the effects of nebulization on the integrity of the DNA-liposome complex and the effect of CF sputum on the efficiency of gene transfer have also been undertaken. A summary of this programme of work will be presented.

References

1 Dorin, J R, Dickinson, P, Alton, E W F W, Smith, S N, Geddes, D M, Stevenson, B J, Kimber, W L, Fleming, S, Clarke, A R, Hooper, M L, Anderson, L, Beddington, R S P and Porteous, D J. 1992. *Nature*, **359**, 211–215.

2 Alton, E W F W, Middleton, P G, Caplen, N J, Smith, S N, Munkonge, F M, Jeffery, P K, Geddes, D M, Hart, S L, Williamson R, Fasold, K I, Miller, A D, Dickinson, P, Stevenson, B J, McLachlan, G, Dorin, J R and Porteous, D J. 1993. *Nature Genetics*, **5**, 135–142.

3 Caplen, N J, Alton, E W F W, Middleton, P G, Dorin, J R, Stevenson, B J, Gao, X, Durham, S R, Jeffery, P K, Hodson, M E, Coutelle, C, Huang, L, Porteous, D J, Williamson, R, and Geddes D M. 1995. *Nature Medicine*, **1**, 39–46.

4 Delaney, S J, Alton, E W F W, Smith, S N, Lunn, P D, Farley, R, Lovelock, P K, Thomson, S A, Hume, D A, Lamb, D A, Porteous, D J, Dorin, J R, and Wainwright, B J. 1995. *EMBO*: in press.

5 Stern, M, Munkonge, F M, Caplen, N J, Sorgi, F, Huang, L, Geddes, D M, and Alton E W F W. 1995. *Gene Therapy* in press

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POSTERS

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Delegates Abstracts

NITRIC OXIDE AND THE RESPIRATORY TRACT IN HEALTH AND DISEASE

Maria G Belvisi
Department of Thoracic Medicine, National Heart and Lung Institute, Imperial College, Dovehouse Street, London SW3 6LY

Nitric oxide is released by a variety of cells following the conversion of L-arginine to nitric oxide (NO) and L-citrulline by the enzyme NO synthase (NOS).[1] NOS is now known to exist in multiple isoforms including constitutive calcium-dependent isoforms present in endothelial cells (eNOS) and some peripheral nerves (nNOS). There is now substantial evidence that NO acts as a neurotransmitter released from inhibitory non-adrenergic non-cholinergic (i-NANC) nerves in human airways, which are the only bronchodilator pathway in humans.[2] Neurally-mediated NANC relaxations in human trachea are associated with a concomitant selective elevation of cyclic GMP, but not cyclic AMP levels, confirming the hypothesis that the NO-cyclic GMP pathway is responsible for mediating the neural bronchodilator response.[3] Furthermore, the pattern of innervation changes throughout the respiratory tract. This is evidenced by a decrease in the density of the nNOS-immunoreactive nerves from proximal to distal airways in agreement with the reduced i-NANC relaxation response in peripheral compared to central airways.[4] Since i-NANC nerves are the only neural relaxant pathway in human airways it is important to determine whether there is any defect in the ability of these nerves to function in diseased airways. In fact, evidence suggests that in tissues from patients with cystic fibrosis i-NANC responses were reduced compared to responses obtained in normal donor tissues with no change in the relaxant action of the nitrovasodilator sodium nitroprusside.[5] If NO functions as an endogenous 'braking' mechanism then this abnormality in the airway i-NANC innervation of cystic patients may lead to exaggerated bronchoconstrictor responses. In fact, this would appear to be the case as a NOS inhibitor enhanced cholinergic neural constrictor responses, with no effect on the contractile response to ACh in human airways *in vitro*. Release of ACh was not affected by NOS inhibitors, indicating that NO modulates cholinergic neural responses by functional antagonism of ACh at the level of the airway smooth muscle.[6] These studies suggest that NO is an important neurotransmitter in the airways and its absence may contribute to the pathophysiology of airways inflammatory diseases.
NOS is also known to exist as an inducible isoform (iNOS) and its activity is increased in acute and chronic inflammation.[7] We have demonstrated that human lung tissue contains significant amounts of Ca^{2+}-dependent NOS activity and that inflammatory diseases such as cystic fibrosis and obliterative bronchiolitis, result in increased amounts of NOS in lung samples.[8] Similarly NOS activity in asthmatic lung (from mild asthmatics) showed a trend to be higher than that in normal lung. Furthermore, higher levels of NOS in asthmatic lung are consistent with previous studies showing that exhaled air from asthmatic patients contains higher amounts of NO than from healthy volunteers.[9] The demonstration that NOS is elevated in airway inflammation provides impetus for continued study of the role of NO in lung disease. However, it is presently impossible to predict whether increased NO formation in pulmonary tissue is associated with tissue damage or protection.

References

1 Moncada, S, Palmer, R M J and Hibbs, E A. 1991. *Pharmacol. Rev.*, **43**, 109–141.
2 Belvisi, M G, Stretton, C D, Yacoub, M H and Barnes P J. 1992. *Eur. J. Pharmacol.*, **198**, 219–221.
3 Ward, J K, Barnes, P J, Tadjkarimi S, Yacoub, M H, Belvisi, M G. 1995. *J. Physiol.*, **483**, 525–536.

4 Ward, J K, Barnes, P J, Springall, D R, Abelli, L, Tadjkarimi, S, Yacoub, M H, Polak, J M, Belvisi, M G. 1995. *Am. J. Cell Mol. Biol.*, **13**, 175–184.
5 Belvisi, M G, Ward, J K, Springall, D R, Buttery, L K D, Tadjkarimi, S, Yacoub, M H, Polak, J M, Barnes, P J. 1994. *Am. J. Respir. Crit. Care Med.*, **149**, A675.
6 Ward, J K, Belvisi, M G, Fox, A J, Tadjkarimi, S, Yacoub, M H, Barnes, P J. 1993. *J. Clin. Invest.*, **92**, 736–743.
7 Vane, J R, Mitchell, J M, Appleton, I, Tomlinson, A, Bishop-Bailey, D, Croxtall, J, Willoughby, D A. 1994. *Proc. Natl. Acad. Sci.*, **91**, 2046–2050.
8 Belvisi, M G, Barnes, P J, Larkin, S, Yacoub, M H, Tadjkarimi, S, Williams, T J, Mitchell, J A. 1995. *Eur. J. Pharmacol.*, **283**, 255–258.
9 Kharitonov, S A, Yates, D, Robbins, R A, Logan-Sinclair, R, Shinebourne, E, Barnes, P J. 1994. *The Lancet*, **343**, 133–135.

HYPOXIA PROLONGS NEUTROPHIL SURVIVAL *IN VITRO*

Edwin R Chilvers
Respiratory Medicine Unit, Department of Medicine (RIE), University of Edinburgh, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW

Neutrophils have an enormous capacity for inducing tissue injury through the release of histotoxic and pro-inflammatory mediators and have been implicated in the pathogenesis of a wide variety of diseases.[1,2] Neutrophil priming and apoptosis represent two of the most important mechanisms controlling the functional responsiveness of these cells to secretagogue agonists. Priming appears to be a prerequisite for neutrophil-mediated tissue injury, whereas apoptosis, through its ability to initiate the uptake of these cells by macrophages, is thought to represent a major mechanism underlying neutrophil clearance from sites of inflammation.[3–5] Our recent studies have demonstrated that a number of agents that prime neutrophils (e.g. GM-CSF, LPS) also delay apoptosis, suggesting a mechanistic link between these two processes.[6] In view of the ability of hypoxia to induce macrophage priming, and reports indicating that reactive oxygen intermediates may initiate apoptosis in certain cells, we have examined the effects of hypoxia on neutrophil survival *in vitro*. This area of study was of additional interest in that neutrophils do not express *bcl-2* and do not undergo apoptosis following activation of respiratory burst activity. The effect of hypoxia on neutrophil behaviour is also relevant to conditions *in vivo* where the oxidant potential at sites in inflammation may be extremely low.

Human peripheral blood neutrophils were purified by dextran sedimentation and plasma/Percoll gradients. Neutrophils (6.75×10^5 cells) were cultured in DMEM containing 10% autologous serum in 21%, 2.5% and 0% oxygen environments and apoptosis assessed morphologically and by DNA electrophoresis. Contrary to expectation, hypoxia caused a major inhibition of apoptosis (% apoptosis 20 h: 78.7±2.2% in 21% O_2, 61.4±6.5% in 2.5% O_2 and 23.1±3.2% in 0% O_2, n=5). No effect of hypoxia was observed at 6 h where the rate of apoptosis was very low (<5%) and hypoxia did not influence cell recovery or viability (trypan blue exclusion). Time-course studies demonstrated <25% apoptosis in neutrophils cultured for 44 h at 0% O_2 compared to virtual total apoptosis/necrosis by 30 h under standard (21% O_2) culture conditions. The inhibitory effect of hypoxia was additive to that induced by GM-CSF (50 U/ml), not associated with induction of *bcl-2* expression, and not mimicked by the antioxidant Trolox (10 mM), superoxide dismutase (200 µg/ml) or methionine (5 mM). Cyclohexamide blocked the effect of hypoxia implying that protection may require protein synthesis. Recent observations have shown that desferrioxamine mimics the effect of hypoxia, implying that this effect may be mediated via a similar hypoxia sensing mechanism to that initiating erythropoietin gene expression in hepatocytes.[7]

These data indicate that hypoxia causes a profound inhibition of neutrophil apoptosis *in vitro*. This effect is directly opposite to the pro-apoptotic effect of hypoxia observed in a number of cell lines and appears not to be mediated via protection against oxygen radical damage. Such an effect of hypoxia on neutrophil survival might delay their removal at sites of inflammation where there is local, and often systemic hypoxia and hence result in additional neutrophil-mediated tissue damage.

References

1 Malech H O *et al.* 1988. *New Eng. J. Med.*, **37**, 687–694.
2 Weiss S J. 1989. *New Eng. J. Med.*, **320**, 365–375.
3 Haslett C *et al.* 1989. *Curr. Opin. Immunol.*, **2**, 10–18.
4 Cox G *et al.* 1995. *Am. J. Respir. Cell. Mol. Biol.*, **12**, 232–237.
5 Savill J S *et al.* 1990. *Nature*, **342**, 170–173.
6 Lee A *et al.* 1993. *J. Leukoc. Biol.*, **54**, 283–288.
7 Maxwell P *et al.* 1993. *Proc. Natl. Acad. Sci., USA.* **90**, 2423–2427.

CHARACTERIZATION OF MOUSE ANTI-EQUINE ALPHA-1-PROTEINASE INHIBITOR MONOCLONAL ANTIBODIES

Mark Dagleish
Department of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG

Alpha-1-proteinase inhibitor (API) is a plasma glycoprotein belonging to the SERPIN (SERum Proteinase INhibitor) superfamily. It is produced in the liver[1] and released into the circulation were by virtue of its molecular weight (53 000 in humans[2]) it can readily diffuse into tissue fluid. The main function of API at its physiological concentration[3] of 3 mg/ml is inhibition of neutrophil elastase,[4] whose uncontrolled activity is thought to be significant in the development of pulmonary emphysema. Equine API is controlled by four closely linked genes (Spi1–Spi4) which are inherited as a single haplotype.[3] There are 22 alleles identified to date.[5]

Equine API extracted from horse serum by affinity chromatography[6] was used to raise mouse anti-equine API monoclonal antibodies based on the method of Kohler and Milstein.[7] Upon repeated cloning out, one cell line was found to be positive for antibodies to equine API when culture supernatant was tested by ELISA. Further analysis by ELISA showed that the antibody was specific for one of the four subtypes of API; Spi1 (specificity to Spi4 was not checked). The antibody is of the IgG class of immunoglobulins.

Maximum supernatant was used to probe various Western Blots. Firstly, this reconfirmed our ELISA results of an Spi1 specific monoclonal antibody. Secondly, the antibody was tested against the native serum proteins of eight common Thoroughbred haplotypes F,G,I,L,N,S,T and U. These were separated by one-dimensional polyacrylamide gel electrophoresis (PAGE) incorporating blue dextran in the stacking gel to retard albumin migration into the resolving gel[8] and transferred to nitro-cellulose by Western Blotting. It was found to be haplotype specific, recognizing Spi1 from I,L,T and U. Thirdly, when tested against a Western Blot produced by one-dimensional sodium dodecyl sulphate (SDS) PAGE in reducing conditions, the antibody was not haplotype specific and recognized all eight forms of Spi1. This suggests that the epitope recognized by the monoclonal antibody is not always accessible when Spi1 is in the native form. However this epitope is present and accessible in all eight haplotypes if the protein is in the reduced state. The next logical step in characterization would be deglycosilation of the Spi1 protein to see if the carbohydrate side chains are responsible for masking the recognized epitope. This is ongoing at present. It is hoped to conclude the characterization of this antibody by developing and varifying methods for its use in immunohistochemistry and immunocytochemistry.

References

1 Perlmutter, D H, Cole, F S, Kilbridge, P, Rossing, T H and Colton, H R. 1985. *Proceedings of the National Acadamy of Science of the USA*, **82**, 795–799.
2 Pannell, R, Johnson, D and Travis, J. 1974. *Biochemistry*, **13**, 5439–5445.
3 Patterson, S D, Bell, K, Shaw, D C. 1991. *Biochemical Genetics*, **29**, 477–499.
4 Beatty, K, Bieth, J, and Travis, J. 1980. *J. Biol. Chem.*, **255**, 3931–3934.
5 Pollitt, C C. and Bell, K. 1983. *Anim. Blood Groups Biochem. Genet.*, **14**, 83–105.
6 Potempa, J, Wunderlich, J K, and Travis, J. 1991. *Biochem. J.*, **274**, 465–471.
7 Kohler, G, Milstein, C. 1976. *Eur. J. Immunol.*, **6**, 511–519.
8 Pemberton, A D, and John, H A. 1993. *Electrophoresis*, **14**, 240–241.

EARLY LIFE INFLUENCES ON INFANT RESPIRATORY ILLNESS AND LUNG FUNCTION

Carol Dezateux
Department of Epidemiology, Institute of Child Health, London WC1N 1EH

Background

It has been suggested that wheezing lower respiratory illnesses (LRI) in infancy are associated with chronic obstructive airway disease in later life.[1] This association may reflect direct damage to the lung at a critical period of lung development, environmental influences, an underlying predisposition to such illness, or some combination of these. While it has been suggested that diminished airway function in the early months of life may predict wheezing LRI in early childhood and reduced lung function at 6 years of age,[2] it is unclear whether these findings are generalizable to populations with differing environmental exposures.

Objectives

To determine whether subtle abnormalities in airway function are present shortly after birth in infants who wheeze in infancy and whether LRI in infancy is associated with impaired airway function at one year of age, after allowing for the effects of growth, environment and pre-morbid lung function.

Design/Setting

A prospective longitudinal population-based study of caucasian term infants recruited from general practices in inner London.

Main outcome measures

Prospective parental report of wheezing episodes and general practitioner diagnosed wheezing in the first year of life; parental report of pre- and post-natal smoking exposure (validated by urinary cotinine assay), family history of asthma and atopy, and other social and medical factors; plethysmographic lung volume, airway resistance and tidal breathing measured on two occasions: at 5–10 weeks (prior to any respiratory illness), and one year of age.

Results

250 infants have been recruited. Respiratory function has been measured on two occasions in over 100 infants whose parents have consented to lung function testing, making this the largest epidemiological study of its kind in Europe. More than half of these infants experienced at least one episode of wheezing in the first year of life and one third of mothers smoked during pregnancy and postnatally. Preliminary analyses have shown that airway function is diminished at one year of age in infants with doctor diagnosed wheezing.[3] Further analyses will examine the association with early life influences and pre-morbid lung function.

References

1 Barker D J P, Osmond C. 1986. *BMJ*, **293**,1271–1275.
2 Martinez F D, Wright A L, Taussig L M, *et al.* 1995. *New Engl. J. Med.*, **332**, 1331–38.
3 Dezateux C, Stocks J, Dundas I, *et al.* 1994. *Pediatr. Pulmonol.*, **18**, 299–307.

MEASUREMENT OF MUCIN SECRETION FROM SHEEP RESPIRATORY GOBLET CELLS BY LECTIN BASED ASSAY

Michael Lethem, Kaicun Zhao, Elizabeth Adam and Udo Schumacher**
Dept of Pharmacy, University of Brighton, Lewes Road, Brighton, BN2 4GJ
**Dept of Human Morphology, University of Southampton, Basset Crescent East, Southampton SO9 3TU*

Respiratory mucus hypersecretion resulting in airway obstruction, particularly of small airways, is a characteristic feature of several respiratory diseases including asthma, chronic bronchitis and cystic fibrosis. In the large airways the major macromolecular constituents of mucus, mucins, are secreted from both submucosal glands and surface epithelial goblet cells, while in the smaller airways, glands are absent and the principal source of mucins are goblet cells. Furthermore, goblet cell hyperplasia is a characteristic feature of hypersecretory conditions and results not only in an increase in the number of goblet cells but also their appearance in distal airways from which they are normally absent. However, most studies of respiratory mucus secretion have employed large airways where, due to the large volume of submucosal glands, the information about goblet cell secretion has been limited. In addition, due to the lack of mucin specific probes, those studies of goblet cells which have been performed have relied on laborious morphometric or videomicroscopic techniques to measure secretory activity.[1–3] We report the development of a simple lectin-based assay for sheep respiratory mucin and its use to monitor the secretory activity of airway epithelial explants in which goblet cells are the sole source of mucin. Histological sections of sheep trachea and airway epithelial explants, prepared as described previously,[4] were screened by lectin histochemistry against a panel of 18 lectins. Several lectins showed specific positive staining of goblet cells and one of these (*Helix pomatia* agglutinin) was incorporated into a microtitre plate assay with a sensitivity of 0–12.5 ng/well of standard mucin. To measure the secretory activity of goblet cells, airway epithelial explants were mounted in Ussing chambers, details of which have been described previously,[4] and perfused on both sides with warmed Ham's F12 containing 15mM sodium bicarbonate and 1mM calcium chloride and equilibrated with 5% CO_2:95% O_2. After an initial equilibration period, samples of mucosal perfusate were collected both during a baseline period and while the cells were exposed to ATP, a known mucin secretagogue. Perfusate samples were subsequently analysed for mucin content by lectin-based assay. Baseline levels of secretion were 13.1±1.9 ng/min/cm^2 (n=6), however bilateral ATP (10^{-4}M) resulted in a rapid five-fold increase in secretion to 65.4±11.9 ng/min/cm^2 (n=6), after which secretory activity returned to near baseline levels over a period of approximately 15 minutes. The identity of the material secreted in response to ATP was confirmed as mucin by CsCl density gradient ultracentrifugation which indicated that the lectin positive material had a density of 1.38–1.53 g/ml, similar to values previously reported for other mucins. These studies indicate that this lectin-based assay, when used in conjuction with airway epithelial explants lacking submucosal glands, is capable of monitoring the secretory responses of sheep respiratory goblet cells. This system is presently being employed in studies of the mechanism and regulation of mucin secretion from respiratory goblet cells with a view to clarifying the role of this cell type in airways obstruction.

References

1 Tokuyama, K, Kuo, H-P, Rhode, J A L, Barnes, P J and Rogers, D F. 1990. *Am. J. Physiol.*, **259**, L108–L115.

2 Kuo, H-P, Rhode, J A L, Tokuyama, K, Barnes, P J and Rogers, D F. 1990 *J. Physiol.*, **431**, 629–641.

3 Davis, C W, Dowell M L, Lethem, M I, and Van Scott, M R. 1992. *Am. J. Physiol.*, **262**, c1313–c1323.
4 Lethem, M I, Dowell, M L, Van Scott, M R, Yankaskas, J R, Egan, T, Boucher, R C and Davis, C W. 1993. *Am. J. Respir. Cell Mol. Biol.*, **9**, 315–322.

INTERACTION BETWEEN HYPOXIC AND CO_2 SENSITIVITY OF ISOLATED SMALL PULMONARY ARTERIES OF THE RAT

Sang-Jin Lee, Niya Xia, John Twaddle and Piers Nye
University Laboratory of Physiology, University of Oxford, Parks Road, Oxford OX1 3PT

The mechanism of excitation of pulmonary vascular smooth muscle bears several striking similarities to the excitation of the carotid body chemoreceptors. Both tissues are excited by hypoxia and by hypercapnia and in both the closure of potassium channels, leading to depolarisation of the cell membrane,[2,3] is widely considered to be central to the process responsible for excitation. It is, therefore, possible that both tissues share a common mechanism for the transduction of hypercapnia and hypoxia into the recorded response.

A key distinguishing characteristic of the behaviour of carotid body chemoreceptors is the multiplicative interaction between the effects of hypoxia and hypercapnia, i.e. CO_2 is a much stronger stimulus in hypoxia than it is in euoxia.[1] We have, therefore, studied the effects of the two stimuli on isolated pulmonary arteries to see if these show multiplicative interaction.

We used a small vessel myograph (Cambustian Ltd, Cambridge, UK) to study 11 small pulmonary arteries (493±29 μ diameter when stretched to a tension equivalent to 25 mmHg transmural pressure). The vessels were preconstricted with PGF2α (1μM). Two levels of hypoxia giving bath PO_2s of *c.* 45 and 35 mmHg, respectively, were used. These both constricted the vessels but did so to markedly different degrees. Once the vessels had been exposed to each PO_2, at a PCO_2 of 30 mmHg, for 40 minutes the PCO_2 was slowly and steadily reduced to 10 mmHg over the course of 30 minutes. All vessels were relaxed as CO_2 was reduced but most had curvilinear responses. Some did not start to relax until PCO_2 had fallen to *c.* 20 mmHg and some stopped relaxing before the lowest PCO_2 was reached, but all had a distinctly straight part of the response curve. We recorded the effect of increasing hypoxia on this straight part and found that at a PO_2 of 45 mmHg the slope was 0.021±0.003mN/mmHg (mean±SEM) while at a PO_2 of 35 mmHg the slope was 0.023±0.003mN/mmHg. There was no significant effect of increasing hypoxia on the slope, i.e. the responses to hypoxia and hypercapnia are not multiplicative, they are merely additive.

Thus hypoxia does not increase the sensitivity of isolated small pulmonary arteries to CO_2 in a way that is qualitatively similar to that observed in the discharge of the carotid body. This suggests that the mechanism underlying the responses of isolated pulmonary vessels is not identical to that acting in the carotid body.

References

1 Fitzgerald, R S and Parks, D C. 1971. Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in rats. *Resp. Physiol.*, **12**, 218–229.

2 Lopez-Barneo, J, Lopez-Lopez, J R, Urena, J and Gonzalez, C. 1988. Chemotransduction in the carotid body: K^+ current modulated by PO_2 in type I chemoreceptor cells. *Science*, **241**, 580–582.

3 Post, J M, Hume, J R, Archer, S L and Weir, E K. 1992. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. *Am. J. Physiol.*, **262**, C882–C890.

RECOMBINANT ADENOVIRUS VECTORS FOR TARGETED GENE DELIVERY

George Santis
Department of Allergy and Respiratory Medicine, United Medical and Dental School of Guy's and St Thomas' Hospitals, Medical School Building, Guy's Hospital, St Thomas Street, London SE1

The host/tissue tropism of recombinant adenovirus vectors is determined by an interaction between the fibre protein and an as yet undefined cell surface receptor. We are attempting to alter the cell binding properties of human adenovirus serotype 5 (hAd5) by genetically modifying its fibre protein, which, in addition to being responsible for binding of hAd5 to its corresponding cell surface receptor, it also contributes to the assembly and/or stabilization of the viral capsid. This function depends on preservation of the trimeric structure of the protein. We have introduced multiple mutations in hAd5 fibre gene and have evaluated the consequence of these mutations on the trimeric structure of the fibre protein and on virus infectivity. Trimerization was determined by expressing the mutated fibre in baculovirus infected Sf9 cells, or by expressing the knob domain of hAd5 fibre in *E.coli*. To assess virus infectivity, we have constructed, for each fibre mutation, a corresponding plasmid vector containing the whole of hAd5 genome (~36Kb), and used these plasmids to infect 293 kidney cells. All viruses were plaque purified, and their identity determined by Southern blot analysis. We found that the introduction of novel *BamHI* restriction sites at nucleotides 31689 (mid shaft region), 31983 (distal shaft region), 32244 (proximal knob region), and 32481 (middle knob region) of hAd5 fibre, the deletion the 294 amino acid residue between nucleotides 31689 and 31983 and the insertion of a flexible peptide linker at the carboxyl terminus (nucleotide 32775) of the protein had no effect on virus infectivity. In contrast, no virus plaques were obtained when the 261 amino acid residue between nucleotides 31983 and 32244, and 237 amino acids between nucleotides 32244 and 32481 were deleted. Analysis of the *in vitro* phenotype of the fibre$^{dd31983\text{-}32244}$ and fibre$^{dd32244\text{-}32481}$ mutated proteins expressed in Sf9 cells showed that both proteins failed to trimerise. The role of the region between nucleotides 32241 to 32481 (this corresponds to the shaft-knod junction of the protein) on fibre trimer formation is being analysed further. We have also attempted to introduce novel ligands in hAd5 fibre. The ScFv for the interleukin 2 (IL-2) receptor (r) cDNA and the IL-2 cDNA were introduced at nucleotides 31689, 31983, 32244 and 32481. However, no viral plaques were obtained after transfection of 293 kidney cells, or of IL-2r expressing cells (Jurkat cells). This would suggest that the expression of a large globular protein in fusion with the fibre protein disrupts the quaternary structure of the protein, and hence virus assembly. We are currently evaluating the consequences of introducing novel petides in fusion with the fibre protein, and we are attempting to delineate the nucleotide sequences in hAd5 fibre that determine trimer formation and participate in the receptor–ligand interaction.

PURIFICATION AND CHARACTERIZATION OF NOVEL NEUTROPHIL ELASTASE INHIBITORS FROM SHEEP AND HUMAN BRONCHOALVEOLAR LAVAGE

Rohit Mistry, Lynne Bingle, Keith Johnson* and Terry Tetley*
Department of Medicine, Charing Cross and Westminster Medical School, London W6 8RF
**Division of Molecular Genetics, University of Glasgow, Glasgow G11 6NU*

A number of inflammatory lung diseases, including emphysema, bronchitis, cystic fibrosis, neonatal and adult respiratory distress syndrome, involve neutrophil influx. Inadequate neutrophil clearance from the site of inflammation and excessive release of active oxygen species and/or proteolytic enzymes can lead to tissue damage. We have focussed on the role of neutrophil elastase (NE) in smoking related emphysema and the endotoxin-induced sheep model of acute lung injury. Apart from its potent action on the extracellular matrix, NE has many pro-inflammatory actions, including complement activation, secretagogue activity, potentiation of metalloproteinase activity and stimulation of IL8 (a neutrophil chemoattractant) secretion. Inherited deficiency of the major serum inhibitor of NE, α1-proteinase inhibitor (PI), predisposes development of emphysema in smokers; this highlights the role of uncontrolled NE in lung tissue damage. Consequently, the action of NE is normally tightly controlled by differential inhibition of the unbound and substrate-bound enzyme. The anti-NE screen of the lung is known to consist of serum-derived PI (54kD) and epithelial cell-derived inhibitors, secretory leukoprotease inhibitor (12kD; SLPI) and elafin (12 and 6kD). These inhibitors are believed to act in concert to inhibit NE in different tissue compartments. However, a significant proportion (up to 40%) of the anti-NE activity in human lung secretions is unidentified and may provide an important part of the pulmonary anti-NE screen. During work on the sheep model of endotoxin-induced acute lung injury, a novel inhibitor of NE was isolated from sheep bronchoalveolar lavage (BAL).[1] It has a molecular mass of approximately 17kD and N-terminal sequencing shows it to be a Kazal-type of protease inhibitor. It has a different combination of properties to either PI or SLPI – it is anionic (SLPI is cationic), inhibits NE and pancreatic elastase (PE; SLPI does not inhibit PE) and is acid stable (PI is not). Furthermore, it accounts for a high proportion of the anti-NE activity of sheep BAL. Antibodies raised to the sheep Kazal inhibitor were used to immunolocalize the inhibitor in sheep lung tissue, where it was found to be in the airway epithelium.[2] Interestingly, during development of acute lung injury, the protein appears to be redistributed, being released into both the airway lumen and interstitium where it may be protective. These data suggest that it is synthesized by secretory epithelial cells and that it is released both apically and basally. Some of the early work on the sheep model involved isolation and characterization of sheep PI,[3] NE and cathepsin G;[4] the molecular mass, partial protein sequencing and complexing with human NE and PI showed that the sheep proteases and antiproteases were highly homologous to the human counterparts. This suggested that the novel sheep Kazal inhibitor could also be a counterpart of a human equivalent which might account for the 'missing' inhibitor in lung secretions. Using the known acid stability, isoelectric point, molecular weight and inhibitory profile of the sheep Kazal inhibitor as a guideline, an inhibitor has been partially purified from human lung lavage; following SDS-PAGE, the human protein migrates in a similar fashion to the sheep inhibitor, with a broad 20kD band and a smaller band of about 6kD. However, its inhibitory profile is different in that it inhibits NE and CG but not PE, whereas the sheep inhibitor inhibits NE and PE but not CG. We are currently further characterizing this inhibitor and cloning both the sheep and human proteins.

References

1 Mistry, R, Snashall, P D, Totty, N, Guz, A, and Tetley, T D. 1992. *Am. Rev. Respir. Dis.*, **145**, A564

2 Mistry, R, Snashall, P D, Lee, C, Bull, T B, Guz, A, and Tetley, T D. 1994. *AJRCCM*, **149**, (part 2), A365

3 Mistry, R, Snashall, P D, Totty, N, Guz, A, and Tetley, T D. 1991. *Biochem. J.*, **273**, 685–690.

4 Mistry, R, Snashall, P D, Totty, N, Guz, A, and Tetley, T D. 1993. *Am. Rev. Respir. Dis.*, **147**, A671

THE RATE OF CHANGE OF FORCED EXPIRATORY VOLUME IN ONE SECOND PREDICTS MORTALITY FROM ISCHAEMIC HEART DISEASE IN THE RENFREW-PAISLEY STUDY

Mark Upton
Departments of General Practice and Public Health, University of Glasgow, Woodside Health Centre, Barr Street, Glasgow G20 7LR

The forced expiratory volume in one second (FEV_1) measures the amount of air exhaled from the lung during the first second of a forced expiration. In epidemiological surveys, subjects with low FEV_1 have an increased mortality not only from respiratory diseases, but also from ischaemic heart disease (IHD) and stroke, even after differences in age, height, cigarette smoking, blood pressure, plasma cholesterol and social class have been adjusted for. It is not yet clear whether these cross-sectional associations between FEV_1 and cardiovascular mortality are causal. This study explores possible associations between the rate of change of FEV_1 and mortality from IHD in middle-aged men and women. An index of FEV_1, percent predicted FEV_1 (%FEV_1), that also takes account of differences between subjects' age and height has been used. Between 1972 and 1976, all residents aged 45–64 years living in Renfrew and Paisley, two towns in the West of Scotland, were invited to participate in a cardiorespiratory survey. A total of 15 411 men and women completed a structured questionnaire and attended a screening examination, a response rate of 78%. 3603 men and 4543 women attended an almost identical examination on average four years later, giving resurvey response rates of 55% and 43% in men and women, respectively. Compared to those who attended, individuals who failed to attend the resurvey but who were alive at the time had a higher prevalence of smoking and were in poorer respiratory health at the time of the baseline survey. The baseline study population has been followed up for cause specific mortality by record linkage. Cross-sectional associations between %FEV_1 measured at baseline and mortality from IHD were observed for resurvey 'attenders' and 'non-attenders' of both sexes. In subsequent analyses, the population attending both surveys has been divided into thirds of the distribution of rate of change of %FEV_1, separately for each sex. Compared to men in the third of the distribution with the slowest rate of change of %FEV_1 between the two surveys, the fully adjusted relative rates of mortality from IHD for men in the middle and fastest thirds were 1.23 (0.95–1.59) and 1.53 (1.19–1.96) respectively. Compared to women in the third of the distribution with the slowest rate of change of %FEV_1 between the two surveys, the fully adjusted relative rates of mortality from IHD for women in the middle and fastest thirds were 1.07 (0.77–1.51) and 1.55 (1.12–2.13) respectively. These estimates have been adjusted for differences in age, baseline %FEV_1, cigarette smoking, systolic blood pressure, plasma cholesterol and social class using the Cox model. In men, there was little change to these estimates after the exclusion either of subjects dying from IHD during the first five years of follow-up or of subjects with pre-existing cardiovascular disease at baseline. It has previously been suggested that factors related to growth in childhood might explain the association between cross-sectional FEV_1 and mortality from IHD. This work suggests that changes to the lung during adult life are important.

CHEMOKINES IN GUINEA-PIG ALLERGIC LUNG INFLAMMATION

Malcolm L Watson
Department of Pharmacology, University of Bath, Bath BA2 7AY

The sensitized guinea-pig is widely used for the study of the mechanisms of allergic airway disease. It exhibits a number of features in common with human asthma, including bronchial hyperresponsiveness, pulmonary eosinophilia and sensitivity to a number of anti-asthmatic drugs. We have previously reported the involvement of the cytokines interleukin (IL) -1 and tumour necrosis factor (TNF) in the airway dysfunction of antigen challenged guinea-pigs.[1] These cytokines induce the release of target cell-selective chemotactic peptides (chemokines) from a number of human airway cell types.[2-3] The aim of our present research is to determine the effects of chemokines on guinea-pig leukocyte activation and airway inflammation, and to assess the profile of chemokine release from guinea-pig lung tissue following cytokine or antigen challenge.

We have tested the activity of a number of human chemokines as stimuli for the elevation of intracellular free calcium ($[Ca^{++}]_i$) and chemotaxis of elicited guinea-pig (gp) peritoneal neutrophils, eosinophils and macrophages. Neutrophil responses to IL-8 and NAP-2 were similar to those obtained with human cells. IL-8 was a weak stimulus of gp eosinophils, but was an effective chemotactic stimulus in IL-5 primed cells. RANTES, which is a good stimulus of human eosinophil $[Ca^{++}]_i$ elevation and chemotaxis, was surprisingly inactive as a stimulus of gp eosinophil responses. In contrast, RANTES was the most effective chemokine tested as a stimulus of gp macrophage $[Ca^{++}]_i$ elevation. Since the poor activity of human RANTES on gp eosinophils could be due to differences between human and gp RANTES peptides, we have purified recombinant gp RANTES from an *E. coli* expression system. This peptide was bioactive in human eosinophil assays with similar activity to human material, and was able to stimulate gp elicited macrophages. However, it was unable to stimulate gp eosinophil chemotaxis, $[Ca^{++}]_i$ elevation or respiratory burst activity.

In order to assess chemokine production by lung cells we have performed northern blot analysis of lung fibroblasts and whole lung tissue. Cultured lung fibroblasts express mRNA for IL-8 and MCP-1 following stimulation with preparations containing guinea-pig TNF activity. Little RANTES mRNA was detected in samples up to 24h following stimulation. Following *in vivo* challenge with aerosolized antigen, MCP1 mRNA was upregulated in lung tissue of ovalbumin sensitized animals compared with non-sensitized animals. RANTES mRNA expression was upregulated 2h after challenge of sensitized animals, although the antigen also increased RANTES expression in naive animals at later time points.

Thus, a role for RANTES in eosinophil recruitment during allergic inflammatory responses in the guinea-pig remains to be determined. MCP1 does seem to be upregulated in the antigen challenged lung, but elicited macrophages are not responsive to this chemokine. Characterization of chemokine activity and production in animal models of disease is important in the understanding of the underlying pathological processes and in the identification of therapeutic targets.

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References

1 Watson, M L, Smith, D, Bourne, A D, Thompson R C and Westwick J. 1993. *Am. J. Resp. Cell Mol. Biol.*, **8**, 365–369.

2 Lukacs, N W, Kunkel, S L, Allen, R, *et al.* 1995. *Am. J. Physiol., (Lung Cell. Mol. Physiol.)* **12**, L856–L861.

3 Standiford, T J, Kunkel, S L, Basha, M A, *et al.* 1990. *J. Clin. Invest.*, **86**, 1945–1953.

SIMPLE METHODS FOR ESTIMATING LUNG FUNCTION – ARE THEY REALLY AS GOOD AS THEY CLAIM?

Jonathan Whiteley
Nuffield Dept of Anaesthetics, Radcliffe Infirmary, University of Oxford, Woodstock Road, Oxford OX2 6HE

Lung volume and dead space volume are critical in estimating the gas exchange efficiency of the human lung. In particular, the distribution of ventilation to lung units of differing volumes is a major factor in determining the effectiveness of gas transfer into and out of the bloodstream. Nitrogen washouts have been used in many attempts to recover ventilation and volumes for multi-compartment lungs. Lewis *et. al*[4] used a fifty-compartment model, from which they claimed to recover the volume and ventilation of each of these compartments. The model ventilates all compartments including dead-space in parallel, with dead-space being defined as a compartment with ventilation but no volume. This model was tested using model lungs by Buchanan *et. al*[1] and in the ICU by Mitchell *et. al*[5] and they were in broad agreement that dead-space volume and ventilation were particularly hard to recover. Wagner[6] used linear programming to investigate the maximum possible ventilation to each compartment given theoretical data, and found that dead-space and units with high ventilation to volume ratios had a very wide range of compatible values. In a theoretical study by Kapitan,[3] it was proved that the nitrogen washout provides only sufficient information for no more than two alveolar compartments with widely seperated time constants and dead-space to be determined. We need to consider the effect of modelling the lung as having a series dead-space, as this volume may be abnormally high for a patient being artificially ventilated. Another technique that we consider is SWIFT (Sine Wave Inspiratory Forcing Technique). A patient being studied using SWIFT is given a small quantity of an inert tracer gas in inspired gas. When equilibrium with this gas is reached, the concentration is perturbed sinusoidally. The mixed- and end- expired gases also oscillate sinusoidally about the same mean value, but with differing amplitudes and phase angles. From these amplitudes and phase angles, estimates of lung parameters are made. This technique is being developed by Hahn[2] and Williams.[7] These authors use a continuous ventilation model, where all lung units including the dead space are modelled as parallel, rigid volumes with a continuous stream of inspired gas entering, and a continuous stream of gas leaving this fixed volume. By using a 'tidal breathing model' we show that the continuous ventilation model is not a particularly accurate approximation to the tidal model, resulting in an underestimate of the dead-space volume and a knock on effect of overestimating the alveolar volume.

References

1 Buchanan, P R, Tavener, S J, Withy, S J, Harris, E A. 1986. *Clin. Phys. Physiolo. Meas.*, 237–253.
2 Hahn, C E W, Black, A M S, Barton, S A, Scott, I. 1993. *J. Appl. Physiolo.*, **75,** 4, 1863–1876.
3 Kapitan, K S. 1990. *J Appl. Physiol.*, **68,** 4, 1621–1627.
4 Lewis, S M, Evans, J W, Jalowayshi, A A. 1978. *J Appl. Physiol.*, **44,** 3, 416–423.
5 Mitchell, R R, Wilson, R M, Sierra, D. 1986. *Int. Journ. Clin. Mon. and Comp.*, **2**, 199–206.
6 Wagner, P D. 1979. *J. Appl. Physiol.*, **46,** 3, 579–587.
7 Williams, E M, Aspel, J B, Burrough, S M L, Ryder, W A, Sainsbury, M C, Sutton, L, Xiong, L, Black, A M S, Hahn, C E W. 1994. *J. Appl. Physiolo.*, **76,** 5, 2130–2139.

CHANGES IN RESPIRATORY CONTROL ASSOCIATED WITH WAKEFULNESS AND SLEEP

Douglas Corfield, Caroline Roberts, Abe Guz , Kevin Murphy and Lewis Adams
Department of Medicine, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF

In man, breathing, both awake and asleep, is stimulated by 'chemical' factors such as increases in PCO_2 and reductions in PO_2. During wakefulness, breathing can also be modified by behavioural requirements such as speech and emotion. Other influences, that change the level of mental arousal, will also modulate wakefulness breathing. However, it is not known what factors determine the level of resting ventilation when awake. We have recently demonstrated[1] that the ability of subjects to voluntarily control their breathing is the same when end-tidal PCO_2 ($PETCO_2$) is held at resting levels (eucapnia) or when it is held about 12 mmHg below eucapnia. However, voluntary control worsens if $PETCO_2$ is increased by as little as 5 mmHg above eucapnia. As the voluntary control of breathing is not 'improved' by reducing $PETCO_2$ below eucapnia it is possible that breathing at rest may be independent of chemical drives. It is hard to test this hypothesis directly for it is difficult to measure chemosensitivity at and below eucapnia. Usually $PETCO_2$ must be reduced by active hyperventilation or by sustained hypoxia; both of which will have undesired effects on such tests. To overcome such problems, we have tested the effects of PCO_2 , around and below eucapnia, on the ventilatory response to a standard peripheral chemoreceptor stimulus using mechanical ventilation via a nasal mask.[2] Subjects were passively hyperventilated (without respiratory muscle activity) at a constant level of ventilation. Stimuli (3–7 breaths N_2) were delivered over a range of steady-state $PETCO_2$ (25–43 mmHg). Stimuli during hypocapnia were coupled with a transient increase in $FICO_2$ so that the stimulus to the peripheral chemoreceptors was always 'hypoxia at eucapnia'. Responses to the stimuli (quantified from the reduction in peak inflation pressure and the magnitude of the evoked diaphragm electromyographic activity) decreased in a graded manner as steady-state $PETCO_2$ fell; the responses disappeared 7.5 mmHg below eucapnia. We concluded that central sensitivity to a peripheral chemoreceptor may be modulated by changes in steady-state PCO_2 around and below eucapnia. These observations would also suggest that changes in PCO_2, even during mild hypocapnia, can potentially modulate awake breathing. Most recently we have applied the same methodology to test changes in respiratory control that occur with sleep. Our initial observations suggest that the response to the peripheral chemoreceptor stimulation is still present during sleep but that the magnitude of the response appears more variable than that present during wakefulness. We speculate that the responses may depend on slight differences in sleep state at the time of each stimulus.

References

1. Roberts, C A, Corfield, D R, Murphy, K, Adams, L and Guz, A. 1995. *Journal of Physiology.* **487**, 107.
2. Roberts, C A, Corfield, D R, Hanson, M A, Calder, N, Adams, L and Guz, A. 1995. *Respiration Physiology* in press.

IMMUNE RESPONSES TO PIGEON MUCIN IN PIGEON BREEDERS' LUNG

Chris Baldwin, Jane Calvert, Anthony Todd and Adrian Allen***
*Department of Immunology and **Department of Physiological Sciences, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH*
**Public Health Laboratory, Cumberland Infirmary, Carlisle, CA2 7HY*

Pigeon breeders' lung (PBL) is a form of extrinsic allergic alveolitis that is caused by the inhalation of avian antigens. The aetiology of the disease is poorly understood but hypersensitivity reactions to pigeon antigens, notably IgA, have been implicated. Precipitating antibodies to pigeon antigens, however, are detectable not only in patients, but also in the sera of some healthy pigeon breeders. We have recently discovered a novel antigen, pigeon intestinal mucin, distinct biochemically and antigenically from previous antigens described including pigeon IgA.[1] Antibodies to mucin were associated specifically with the sera from pigeon breeders and like the antibodies to pigeon IgA, were found in both symptomatic and asymptomatic individuals. When the IgG subclass composition of these antibodies was examined in precipitin positive pigeon breeders, IgG1 antibodies to pigeon IgA and both IgG1 and IgG2 antibodies to pigeon mucin were demonstrated in all sera (from both symptomatic and healthy breeders). However, while IgG3 antibodies to mucin were found in all symptomatic individuals, only very weak IgG3 reactivity to this antigen was occasionally observed in asymptomatic individuals.[2] No significant IgG2 and IgG3 antibodies to pigeon IgA were demonstrated in pigeon breeders.

Pigeon intestinal mucin is a large molecular weight glycoprotein (over 80% by weight carbohydrate) similar in structure to the well characterized mammalian gastrointestinal mucins. Studies suggest that the epitopes of pigeon intestinal mucin reacting with human PBL antibodies are primarily carbohydrate in nature and these epitopes are not present on human gastrointestinal mucins. Studies by others have shown pigeon secretory IgA is an antigenic component in pigeon feather bloom and pigeon droppings. Our recent studies have now shown the presence of mucin antigen in both pigeon bloom and droppings.

Our current work focuses on the quantification of the IgG subclass responses to avian mucin in exposed, asymptomatic, pigeon breeders. 27/93 of the sera screened had significantly high levels of IgG3 to mucin when compared to healthy unexposed individuals. In almost all cases (25/27) IgG3 was a minor component, less than 7%, of the total anti-mucin antibody response while IgG1 and/or IgG2 where the major isotypes. IgG1 and IgG2 responses to avian mucin in individual sera showed a positive correlation between antibody affinity and concentration ($r = 0.79$ and $r = 0.73$ respectively). No such correlation was observed for IgG3 responses ($r = 0.33$) in individual sera suggesting that the concentration and affinity of anti-mucin IgG3 may be related to disease progression.

References

1. Todd, A, Coan, R M and Allen, A. 1991 Pigeon breeders' lung: pigeon intestinal mucin, an antigen distinct from pigeon IgA. *Clin. Exp. Immunol.*, **85**, 453–458.
2. Todd, A, Coan, R. and Allen, A. 1993 Pigeon breeders' lung; IgG subclasses to pigeon intestinal mucin and IgA antigens. *Clin. Exp. Immunol.*, **92**, 494–499.

VENTILATORY RESPONSE TO HYPOXIA IN NORMOXIC AND CHRONICALLY HYPOXIC POST-NEONATAL RATS

Estelle Moore and Denise Bee
Department of Medicine and Pharmacology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

An attenuation in the hypoxic ventilatory response (HVR) has been observed at *c.* 5 weeks of age in the normoxic neonatal rabbit.[1] A loss in sensitivity of the peripheral chemoreflex possibly due to an elevation in the levels of the inhibitory catecholamine, dopamine, within the carotid body could explain this phenomenon. Dopamine levels are elevated in the carotid bodies of some victims of Sudden Infant Death Syndrome (SIDS)[2] and may be linked to the characteristic sudden failure of respiration the peak of which occurs around four months of age.
Wistar rats born and reared in a normobaric chamber (F_iO_2, 0.1) were compared to normoxic (N) rats. Every two days, for twenty days, awake rats (CH, N) were weighed and their ventilation (V_E), while breathing varying F_iO_2 (0.3, 0.1, 1.0), was measured using head-out plethysmography. Basal ventilation (V_E) was taken as ventilation at F_iO_2 of 0.3 (ml/min). The HVR was taken as the percentage change in V_E (% ΔV_E) from F_iO_2 0.3 to 0.1.

Table 1. Basal ventilatory and hypoxic ventilatory response data in post neonatal rats (means ± sem)

Age (days)	10	12	14	16	18	20
Normoxics (n=6)						
Weight (g).	17.0±0.3	21.3±0.2	24.9±0.4	27.9±0.3	30.8±0.3	37.0±0.5
V_E (ml/min).	31.3±3.0	40.5±6.0	28.7±2.6	74.2±10.2*	44.8±8.7	39.1±3.3
% Δ V_E	49.1 ±17.2	55.6 ± 21.1	50.9 ± 13.8	-2.15 ± 6.3*	5.74±8.5*	37.4±10. 9
Chronic Hypoxics (n=17-6)						
Weight (g)	12.5±0.3#	14.1±0.6#	16.2±0.9#	17.5±0.8#	18.2±1.4#	21.5±2.0#
V_E (ml/min)	17.5±1.6*	44.5±5.2	46.2±2.8	31.7±3.0*	64.4±12.2	63.1±8.7
% ΔV_E	43.3±8.1	1.3±7.2*	10.8±7.8	12.9±4.1	10.9±9.7	25.3±7.7

*P < 0.01 ANOVA within group comparison; between N and CH @ same age,
#P < 0.05 unpaired Student t-test.

The body weight of CH rats was significantly lower than normoxics on each day of the study. In N rats the HVR was significantly attenuated at day 16 and this coincided with a significant increase in basal V_E. The basal V_E recovered but the HVR remained depressed until day 18 after which this also appeared to recover. CH rats gave highly variable results but as a group showed an earlier but similar pattern to N rats (day 12) but with a continuing depression of HVR (12–18 days); basal V_E also remained high.

In summary we observed that post-neonatal rats showed an attenuation of their ventilatory response

to hypoxia which was more prolonged and occurred earlier in CH than N rats. The mechanism of this loss in hypoxic sensitivity of the peripheral chemoreflex at such a specific time in post-neonatal normal development remains unclear but an investigation into a possible causative role of dopamine is currently being investigated. Abnormalities in respiratory control have been suggested as an aetiology for SIDS. However should a normal loss of HVR occur in human babies similar to that seen in rats this could be a period of high risk during which additional stresses (or even just sleep), that involve hypoxic episodes, could lead to terminal events. In addition this pattern of development, being a normal phenomenon, might explain the age clustering of SIDS.

References

1 Bee, D, Wright, C and Pallot, D J. 1993. *J. Physiol.*, **467**, 283P.

2 Perrin, D G, Becker, L E, Madapallimatum, A, Cutz, E, Bryan, A C, Sole, M J. 1984. *The Lancet*, Sept. 8, 535–537.

HLA AND TCR RELATED GENES AND ATOPY AND ASTHMA

Miriam F Moffatt and William O C M Cookson
Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

The type of allergen to which atopic individuals react is of clinical importance, as House Dust Mite (HDM) allergy is associated with an increased risk of asthma and bronchial hyper-responsiveness, compared to grass allergy. Candidate genes influencing specific allergy include the HLA Class II genes and the genes of the T Cell Receptor (TCR). We have previously demonstrated genetic linkage of specific IgE responses to the TCR-α/δ locus on chromosome 14, in independent British and Australian populations (850 subjects in total).[1] We have now HLA-DRB typed these subjects and have typed them for a biallelic polymorphism in the Vα8.1 gene of the TCRα/δ locus.

Australian subjects have had significantly higher levels of exposure to HDM than the British, and have four-to-five fold higher IgE titres to *Der p* I and *Der p* II. Titres of *Fel d* I are similar in the two populations. Allele frequencies for HLA-DRB and for the Vα8.1 polymorphism are significantly different in the two populations.

In Australians, the level of IgE response to *Der p* I was increased in subjects who had Vα8.1 allele 2, and were HLA-DRB1*02 positive (p=0.004): similar results were seen with *Der p* II responses (p=0.006). This result was not seen in the British population. A further set of 420 Australian subjects have been examined. Preliminary results confirm the Vα8.1 allelic association with responses to HDM.

Allelic associations with IgE responses to other major allergens have also been seen for the two microsatellite markers, FCA.TA1 and D14S50, used in the original genetic linkage study.[1] These associations may be due to linkage disequilibrium with as yet unknown polymorphisms in the genes of the TCR-α/δ locus. The association between *Der p* I and *Der p* II and the Vα8.1 polymorphism however, could be causal. Investigation of other Vα polymorphisms may identify further associations with other major allergens.

Genomic polymorphisms in the TCR-α/δ region may, therefore, restrict IgE responses to particular antigens. This restriction may be in the context of particular HLA-DR types. These genetic effects are modified by the environment.

We have also tested for associations with other MHC polymorphisms, notably near the TNF genes. We find associations of a *Nco I* polymorphism with asthma in Australian and English subjects (Odds Ratio 1.72, 95% CI 1.37-2.16, p=0.0000), as suggested by Campbell and Morrison *(personal communication).*

Reference

1 Moffatt, M F, Hill, M R, Cornélis, F, *et al.* 1994 *The Lancet*, **343**, 1596–1600

COMPARISON OF PALATAL MUSCLE REFLEX ACTIVITY IN SLEEP APNOEA PATIENTS AND NON-SNORING SUBJECTS

Ian L Mortimore and Neil J Douglas
Respiratory Medicine Unit, Department of Medicine, University of Edinburgh, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW

The sleep apnoea/hypopnoea syndrome (SAHS) causes daytime sleepiness and impaired cognitive function in 1–4% of the middle-aged population and is associated with an increased frequency of road traffic accidents, myocardial infarction and stroke.[1] The syndrome results from repeated occlusion of the upper airway during sleep, but it is unclear what determines whether an individual's airway will obstruct. Recent attention has focused on upper airway size with some,[2] but not all,[3] studies showing narrower upper airways in awake SAHS patients compared to normal subjects. However, even the studies showing a difference demonstrates considerable overlap in airway size between normal subjects and SAHS patients. An alternative hypothesis is that SAHS patients exhibit differences in the neuromuscular control of their upper airways. In normal subjects, the intrinsic tongue muscle genioglossus[4] and the soft palatal muscles levator palatini and palatoglossus[5] tense with each inspiration and exhibit reflex activity in response to negative pressure. Furthermore, the reflex activity of genioglossus in normal subjects is known to be reduced during sleep.[4] These characteristics may be important in the maintenance of upper airway patency, as collapse in SAHS patients is known to occur usually at the retropalatal or retroglossal level.[6] We have, therefore, compared the reflex responses of the antagonist palatal muscles levator palatini (LP) and palatoglossus (PG) to different levels (0, -2.5, -5, -7.5, -10, and -12.5 cm H_2O) of 'square wave' negative pressure application in 16 awake non-snoring male subjects and 16 awake male SAHS patients using electromyography (EMG). Results were expressed as percentage maximum EMG activity. In normal subjects, both LP ($p<0.001$) and PG ($p<0.001$) demonstrated increasing EMG activity in response to increasing negative pressure applied via the nose or mouth (reaching 80% maximum at -12.5 cm H_2O). In SAHS patients, LP demonstrated a significant increase in activity in response to negative presure applied via the nose ($p=0.006$) or mouth ($p=0.04$), reaching 46% and 57% respectively at -12.5 cm H_2O. PG showed only a non-significant trend to increasing activity with increasing negative pressure when applied via the nose ($p=0.17$) and mouth ($p=0.19$), 41% and 51% respectively at -12.5 cm H_2O. SAHS patients demonstrated a significant reduction in response to negative pressure application compared to normal subjects for both LP ($p<0.001$) and PG ($p<0.001$) irrespective of the route of negative pressure application (mouth $p<0.001$; nose $p<0.001$). We conclude that the reduced reflex response of LP and PG to negative pressure application in patients with SAHS may contribute to the pathogenesis of upper airway collapse and thus obstructive apnoeas.

References

1 Douglas, N J, and Polo, P. 1995. *The Lancet*, **344**, 653–655.
2 Brown, I G, Bradley, T D, Phillipson, E A, *et al.* 1985. *Am. Rev. Respir. Dis.*, **132**, 211–215.
3 Hoffstein, V, Wright, S, Zamel, M, *et al.* 1991. *Am. Rev. Respir. Dis.*, **143**, 1294–1299.
4 Horner, R L, Innes, J A, Morrell, M J, *et al.* 1995. *J. Physiol.*, **476**, 141–151.
5 Mortimore, I L, Mathur, R and Douglas, N J. 1995. *J. Appl. Physiol.*, **79**, 448–54.
6 Morrison, D L, Launois, S H, Isono, S, *et al.* 1993. *Am. Rev. Respir. Dis.*, **148**, 606–11.

AMBULATORY MEASUREMENT OF END-TIDAL P_{CO_2} IN RESPIRATORY MEDICINE

William Gardner and John Varley
Department of Thoracic Medicine, King's College School of Medicine and Dentistry, Bessemer Rd, London SE5 9PJ
Human Biology, School of Medicine, The University, Leeds

Most respiratory research in conscious humans relies on measurements via a mouthpiece or other invasive devices. In that breathing can be controlled via both cortical and automatic pathways (itself a very understudied area of research), these measurement devices may both modify the breathing being studied, and limit recordings to short periods in a laboratory. There is a need for uninvasive measurements over long periods of time and during a subject's normal activities similar to the 24 hour ambulatory ECG recordings which are indispensable in cardiology. End-tidal measurement of PCO_2 is well established and, in awake subjects with normal lungs and in the absence of heavy exercise, is close to arterial PCO_2. We have repeatedly demonstrated the feasibility of prolonged recording from a fine sampling catheter taped just inside one nostril. Varley[1] has developed a portable capnograph linked to a small telemetry transmitter which can be worn by the patient, sampling via a moisture permeable catheter. The mean rise time from 10-70% maximum response to a step of CO_2 is 99 ms, fast enough for end-tidal sampling up to moderate respiratory rates. The telemetry range is 50 yards indoors, 150 yards out of doors, and the signal is received by a stationary receiver/computer unit which can be located in a central position in a patient's home. The unit operates for about 12 hours off a set of three small lithium batteries. This system has been linked to our online computer program with A-D sampling at 100 Hz to provide a measure of $PETCO_2$, slope of the end-tidal plateau and breath timing for every breath for up to 12 hours. There are many potential uses for this system, to be discussed in this forum. We first need to define the range of $PETCO_2$ in normal subjects during everyday activities as there is considerable uncertainty about the lower limit of this range. We then want to study the difficult group of patients with spontaneous hyperventilation as frequently encountered by chest physicians. Our views on this subject, and all references are contained in a review in press.[2] Hyperventilation indicates excessive respiratory drive which can have many causes. In the clinical situation, these causes need to be identified, the sole diagnosis of 'hyperventilation syndrome' being, in our view, inadequate. Based on extensive clinical experience we routinely identify six different clinical situations in which hyperventilation can contribute to the presenting symptomatology, these groups having implications for prognosis and management. Our original description was of patients with chronic hyperventilation and we now believe that this is a different group from patients with more acute hyperventilation, sustained by a combination of factors but especially misattribution to serious disease. Dyspnoea is not necessarily synonymous with hyperventilation, but 'air hunger', or difficulty breathing-in, is common but little studied, may be of psychogenic origin and leads to both panic and hyperventilation. Disproportionate dyspnoea on exertion with no detectable etiology is also common; it causes variable hyperventilation, and has defied all our attempts to provide an explanation. Finally organic disorders such as asthma can occasionally present with the symptoms of hypocapnia. Why asthmatics should present in this way is unclear. Primary anxiety in isolation is rarely a cause of hyperventilation in any of these groups. The longest recording of $PETCO_2$ that we have so far made in any of these groups is an hour. To characterize these groups in more detail, we need to record for long periods and ideally during controlled and routine activities in the patients' own environment.

References

1 Varley, J S, The design and evaluation of portable instrumentation for ambulatory respiratory CO_2 measurement. University of Leeds: 1994.

2 Gardner, W N. The pathophysiology of hyperventilation related disorders. *Chest.* Review - *in press,* 1995.

RED NUCLEUS MEDIATES THE BIPHASIC RESPIRATORY RESPONSE IN NEONATES

Gareth L Ackland
Department of Obstetrics and Gynaecology, University College London Medical School, 86–96 Chenies Mews, London WC1E 6HX

Apnoea and respiratory failure are major causes of morbidity and mortality in the newborn. One component of this problem is that the respiratory response to isocapnic hypoxia is biphasic, especially in the newborn; the initial increase in ventilation produced by stimulation of the peripheral arterial chemoreceptors is followed, after a few minutes, by a decline towards control. This fall in ventilation is not due to arterial chemoreceptor adaptation or failure and, in fact, additional transient stimulation of the chemoreceptors fails to elicit reflex effects on breathing during this secondary phase of falling ventilation.[1] Thus during the secondary phase, increased chemoreceptor discharge is prevented from exerting effects on respiratory output. For many years this biphasic respiratory response has been attributed to the operation of a CNS descending mechanism which inhibits respiratory output (RO). It may represent a persistence of the powerful inhibition of breathing movements which occurs in hypoxia in the fetus. In both fetus and neonate, brain stem transection studies indicate that the mechanisms involve structures at the level of the upper pons/midbrain, but no clear structure was targetted. We pursued this by using 27±1 day old rabbits, an age at which they show a biphasic respiratory response, studied after decerebration to avoid the confounding effects of anaesthesia. RO was measured from phrenic efferent activity. Initially we used electrical stimulation to identify areas in the brain stem from which an inhibition of RO in normoxia could be evoked. These studies confirmed that this is possible from several structures in the pons (including locus coeruleus, raphe nuclei, pontine reticular nuclei) but a most powerful effect was evoked from a discrete site in the midbrain which histology revealed to be the red nucleus (RN). This confirms earlier studies in the adult.[2] Subsequently we found that bilateral lesions in the RN abolished the fall in RO in hypoxia, without affecting breathing in normoxia or the effects of hypoxia on arterial blood pressure. These studies did not allow us to discriminate between two possibilities, *viz* that RN cells mediate the fall in RO, and that the response is mediated by axons passing through this area. To address this issue, we performed microinjections of glutamate to excite cells but not axons, in the RN and adjacent areas of the midbrain. Glutamate produced a fall in RO at RN sites where electrical stimulation evoked apnoea, and produced either no effect or a stimulation of RO at sites outside the RN at which electrical stimulation similarly produced no effect or stimulated RO. This makes it clear that cells in the RN mediate the fall in RO in hypoxia in the neonate. This provides an important advance as it gives a target for future investigations. For example, we do not know the process by which the RN cells initiate the inhibitory effect – are they sensitive to hypoxia or do they receive an input from elsewhere, e.g. the peripheral arterial chemoreceptors or the RVLM? Nor do we know the pathway by which the inhibition occurs – is it via a di- or pauci-synaptic effect on phrenic motoneurones in keeping with other influences of the RST[2,3] or via a relay in the pons? Lastly, we know that respiratory chemoreflexes are supressed in some infants at risk of respiratory failure, but we do not know whether an abnormality of the RN mechanism contributes to this condition.

References

1 Ackland, G L, Moore, P J and Hanson, M A. 1994. Is the ventilatory decline seen in newborns during hypoxaemia centrally mediated? In *Arterial Chemoreflexes (Cell to System). Advances in Experimental Medicine and Biology*, **360**, 345–348. O'Regan, R G, Nolan, P, McQueen, D S, and Paterson, D J (Eds). Plenum Press.

2 Schmid, K, Bohmer, G and Fallert, M. 1988. Medullary respiratory-related neurons with axonal connections to rostral pons and their function in termination of inspiration. *Pflugers Archiv.*, **403**, 58–65.

3 Keifer, J and Houk, J C. Motor function of the cerebellorubrospinal system. 1994. *Physiol. Rev.* **74**(3), 509–542.

NUCLEOTIDE REGULATION OF INTRACELLULAR CALCIUM AND ION CHANNEL ACTIVITY IN HUMAN NORMAL AND CYSTIC FIBROSIS LUNG

Deirdre Walsh, Valérie Urbach and Brian Harvey
Wellcome Trust Cellular Physiology Research Unit, Department of Physiology, University College Cork, Republic of Ireland

In cystic fibrosis (CF), mutations in the CF gene product CFTR (a cAMP-regulated chloride channel), cause a defect in chloride secretion and other transport abnormalities.[1] Hyper-absorption of sodium is associated with decreased chloride secretion in the lung proximal airway epithelium. The CFTR protein may also be a channel regulator, controlling the activity of an outwardly rectifying Cl^- channel (ORCC) [2] and the Na^+ channel.[3] Extracellular nucleotides such as uridine triphosphate (UTP) activate ORCC in airway epithelia. The ORCC is activated by calcium (>100 nM)[4] in excised inside-out apical membrane patches. We have found intracellular calcium ($[Ca^{2+}]_i$) to be increased in normal lung and in CF (ΔF508 mutation) trachea epithelia upon exposure to extracellular UTP.

In normal lung, UTP (100 μM) increased $[Ca^{2+}]_i$ from 250±11 to 1150±36 nmol/l. In CF trachea, UTP increased $[Ca^{2+}]_i$ from 65±14 nmol/l to 400±10 nmol/l (n = 6, mean ± SEM). The effects of UTP on $[Ca^{2+}]_i$ were half-maximal at [UTP] = 10 μM and lasted 30 minutes for a 4 minute exposure. Release of calcium from intracellular stores (endoplasmic reticulum) appears to be the mechanism for the rise in $[Ca^{2+}]_i$ since the effect of nucleotide was apparent in the absence of extracellular calcium. Adenosine triphosphate (ATP) was equipotent with UTP in mobilizing intracellular calcium. The nucleotide order of potency UTP=ATP>ADP>>AMP indicates the presence of a P_{2u}-type purinergic receptor on the luminal membrane.

ATP may act locally on purinergic receptors if secreted by the lung epithelium. There is electrophysiological evidence[5] that the CFTR protein may transport ATP. We have tested the effects of activation of CFTR by forskolin in normal human lung epithelium and found forskolin caused an increase in $[Ca^{2+}]_i$ which could be inhibited by hexokinase (consumes extracellular ATP by phosphorylation of D-glucose). These data indicate that ATP release via CFTR can act as an autocrine regulator of $[Ca^{2+}]_i$ in human lung. Sodium absorption in sheep trachea[6] has been shown to be decreased by experimental manoeuvres designed to cause an increase in $[Ca^{2+}]_i$ concentration. Extracellular ATP and UTP, via effects on $[Ca^{2+}]_i$, may, therefore, have therapeutic potential to simultaneously stimulate chloride secretion while down-regulating sodium hyper-absorption in CF airway epithelia.

References

1 Knowles, M R, Stutts, M J, Spock, A, Fisher, N, Gatzy, J T and Boucher, R C. 1983. *Science*, **221**, 1067–1070.
2 Schwiebert, E M, Egan, M E, Hwang, T H, Fulmer, S B, Allen, S S, Cutting, G R, and Gugggino, W B. 1995. *Cell*, **81**, 1063–1073.
3 Stutts, M J, Canessa, C M, Olsen, J C, Hamrick, M , Cohn, J A, Rossier, B C, and Boucher, R C. 1995. *Science*, **269**, 847–850.
4 Urbach, V, Prosser, E, Raffin, J P, Thomas, S and Harvey, B J. 1994. *J. Gen. Physiol.*, **104**, 84.
5 Paysk, E A, and Foskett, J K. 1995. *J. Gen. Physiol.*, **105**, P53.
6 Graham, A, Steel, D M, Alton, E W, Alton, F W and Geddes, D M. 1992. *J. Physiol.*, **453**, 475–491.

MECHANISMS INVOLVED IN NEUTROPHIL RECRUITMENT IN PULMONARY INFLAMMATION

S Terry Woolley and Paul G Hellewell
Applied Pharmacology, National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, Dovehouse Street, London SW3 6LY

The rapid recruitment of neutrophils to the lung is a feature of diseases such as bacterial pneumonia and the adult respiratory distress syndrome (ARDS). While the molecular mechanisms of neutrophil recruitment from post-capillary venules in the systemic peripheral circulation (e.g. skin) are reasonable well understood, how neutrophils sequester in pulmonary capillaries during lung inflammation and how they migrate into the airspaces is less clear.

We have investigated the process of migration in an experimental model of pulmonary inflammation in the rabbit and compared neutrophil recruitment at this site with recruitment in the skin of the same animals. Neutrophil migration into the airspaces is induced by the intrabronchial instillation of chemoattractant molecules (C5a, IL-8), cytokines (IL-1α) or mediators generated endogenously in response to LPS or immune complex deposition.[1,2] The same stimuli are injected intradermally and neutrophil recruitment assessed by measuring the accumulation of radiolabelled (^{111}In) neutrophils (in lavage fluid and in skin sites) and total neutrophils (in lavage). Neutrophil recruitment to the airspaces induced by C5a or IL-8 is independent of the leukocyte 2 integrin CD18 as assessed using blocking mAbs; in contrast, neutrophil recruitment to the skin of the same animals is totally CD-18-dependent.[1] Other agents which clock recruitment in the skin also have no effect on neutrophil migration into the airspaces.[3] Accumulation of neutrophils in the airspaces after IL-1, LPS or immune complexes is partially (~50%) CD18-dependent[2] leading us to speculate that under normal circumstances capillary endothelial expression of ICAM-1 (a major ligand for CD18) is low or insignificant, but it is upregulated by certain agents such that migration then becomes CD18-dependent. Investigations using a radiolabelled anti-ICAM-1 mAb to quantify endothelial ICAM-1 expression *in vivo* to support this idea.

These data suggest the existence of a major CD18-independent pathway for neutrophil migration from pulmonary capillaries. It is difficult to envisage a role for selectins in this process since the selectin-mediated rolling that occurs in post-capillary venules is unlikely to occur in the smaller capillaries. Nevertheless, using a radiolabelled anti-E-selectin mAb we have found that E-selectin is rapidly (1 hour) upregulated on pulmonary capillaries by LPS, but not by C5a,[4] although we have yet to establish the significance of this finding. The role of L- and P-selectin has been studied using fucoidin, a polysaccharide which is a functional blocker of these selectins *in vivo*. Neutrophil accumulation to C5a and LPS in skin was abolished by fucoidin demonstrating the efficacy of this molecule. In the same animals, neutrophil recruitment to the airspaces induced by either agent was unaffected indicating that L- and P-selectin are unlikely to be involved in migration.[5]

These data show that neutrophil recruitment in pulmonary inflammation occurs by mechanisms largely distinct from those that operate in post-capillary venules of the systemic circulation. A better understanding of these mechanisms will become critical in selecting agents to decrease neutrophil accumulation in the lung and targeting those diseases in which neutrophil influx is a major characteristic.

References

1 Hellewell, P G, Young, S K, Henson, P M and Worthen, G S. 1994. *Am.J. Respir. Cell Mol. Biol.*, **10**, 391–398.

2 Fairbairn, S M, Norman, K E, Woolley, S T, Jeffery, P K, Rossi, A G and Hellewell, P G. 1996. Submitted.

3 Hellewell, P G, Young, S K, Henson, P M and Worthen, G S. 1995. *Am.J. Resp.Crit.Care Med.*, **151**, 1218–1227.

4 Woolley, S T, Wolitzky, B A and Hellewell, P G. 1996. Submitted.

5 Woolley, S T, Fairbairn, S M, Norman, K E, Rossi, A G and Hellewell, P G. 1996. Submitted.

EXPRESSION OF NORMAL AND MUTANT *CFTR* FROM YEAST ARTIFICIAL CHROMOSOMES

Clare Huxley
Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine, Norfolk Place, London W2 1PG

The outstanding problems of gene therapy are efficient delivery, high levels of tissue specific gene expression, and long-term maintenance and expression of the input DNA. The problem of full levels of expression is well demonstrated in transgenic mice where minigene constructs are often very poorly expressed and the tissue specificity of expression is dependent on the exact promoter used. Our strategy is to start with much larger genomic constructs that should contain all the long range controlling elements such as locus control regions to give full levels of tissue specific and controlled expression.

Transgenic mice carrying the human *CFTR* gene were made by microinjection of a 310 kb Yeast Artificial Chromosome which contains the intact gene spanning about 230 kb and also about 80 kb of upstream DNA. These transgenic mice have been crossed with the *CFTR* knock-out mice made in Cambridge[1] to give mice which only express the human transgene. In one of the lines, the YAC DNA is intact and the human *CFTR* gene complements the null phenotype and appears to be expressed at about the same levels and in largely the same tissues as the mouse gene. This indicates that the YAC contains all the DNA necessary for high levels of tissue specific expression suitable for use in gene therapy applications.

Delivery of DNA for gene therapy is generally inefficient and is not feasible at the moment with fragments of DNA hundreds of kb in size. We are making deletion variants of the YAC which still drive full levels of tissue specific expression but which are small enough for efficient delivery. The variants will lack various numbers of introns and have a reduced amount of upstream DNA and will be made using homologous recombination in the yeast host. These constructs will be introduced into cells in tissue culture and into transgenic mice where the levels of expression will be assayed.

The mice that we have generated so far express only the normal human *CFTR* gene. We are now making constructs that will express only a mutated form of the human *CFTR* gene at full levels and in a tissue specific manner. The mutation we have chosen to make is R117H which generally causes a mild phenotype with pancreatic sufficiency. In addition, the severity of the phenotype correlates with the number of Ts in the splice acceptor in intron 8 which in turn correlates with the efficiency of splicing of exon 9. Both the R117H mutation and the different T haplotypes in intron 8 are being introduced into the YAC by homologous recombination. These modified YACs will then be introduced into transgenic mice to test the relationship between the R117 mutation and the variable number of Ts with the splicing efficiency and CF pathology in the mice.

Reference

1 Ratcliff, R, Evans, M J, Cuthbert, A W, MacVinish, L J, Foster, D, Anderson, J R, and Colledge, W H. 1993. *Nature Genetics*, **4**, 35–41.

REGULATORY SEQUENCE ABNORMALITY OF THE ALPHA-1-ANTITRYPSIN GENE IS ASSOCIATED WITH CHRONIC RESPIRATORY DISEASE DUE TO A DEFECTIVE INTERLEUKIN-6 RESPONSE

Peter Marsters, Kevin Morgan, Graeme Scobie and Noor Kalsheker
Department of Clinical Laboratory Sciences, Division of Clinical Chemistry, Queen's Medical Centre, Nottingham NG7 2UH

Chronic respiratory disease affects about 3% of the population in western countries[1] and is expected to cause increasing morbidity associated with an ageing population. About 20% of patients with chronic respiratory disease have a familial component that is predominantly genetic in origin.[2] Alpha-1-antitrypsin deficiency (AATD) leads to progressive lung damage in early adult life in cigarette smokers.[3] AATD only accounts for about 2% of all patients with chronic respiratory disease.[4] Alpha-1-antitrypsin protects the lower respiratory tract from damage by neutrophil elastase.[5] We have identified a mutation in the 3' flanking sequence of alpha-1-antitrypsin that occurs in about 17% of patients with chronic respiratory disease and about 5% of the general population ($p = 0.0016$).[5] The mutation is a single base substitution that occurs in a regulatory sequence.[6] We have demonstrated specific binding of nuclear proteins by gel-shift analysis and DNase I footprinting and increased *in vivo* transcriptional activity by transfection of mammalian cells containing DNA fragments corresponding to the region of interest.[6] In contrast, the mutant sequence demonstrated loss of binding to nuclear proteins and reduced transcriptional activity.[6] We have demonstrated that the mutation affects binding of a specific transcription factor of the C/EBP family. Transcription factors of the C/EBP family play a central role in mediating the effects of interleukin-6, a cytokine which regulates the acute-phase response. The mutation does not appear to affect basal expression, as the plasma concentration of alpha-1-antitrypsin protein is normal in individuals who carry it. It may, however, reduce the three- to four-fold rise in plasma concentrations that occurs during inflammation/ We have demonstrated that the transcription factor, octamer-1 protein, specifically binds to wild-type sequence in this region, but mutant sequence binds poorly. With wild type sequence there is a co-operative interaction between octamer-1 protein and NF-IL6, a member of the C/EBP family, and this co-operativity is dramatically reduced with mutant sequence. These studies highlight a novel mechanism of disease related to a diminished acute-phase response.

References

1 Hay, J W and Robin, E D. 1991. *Am. J. Public. Health.*, **81**, 427–433.
2 Cohen, B H, Ball, W C and Bias, W B. 1975. *John Hopkins Med. J.*, **137**, 94–104.
3 Crystal, R G. 1990. *Clin. Invest.*, **85**, 1343–1352.
4 Mittman, C, Barbela, T and Liebermann, J. 1973. *J Occup. Med.*, **15**, 33–38.
5 Kalsheker, N A, Hodgson, I J, Watkins, G L, White, J P, Morrison, H M and Stockley, R A. 1987, *Br. Med. J.*, **294**, 1511–1514.
6 Morgan, K, Scobie, G and Kalsheker, N A. 1993. *Hum. Mol. Genet.*, **2**, 253–257.

THE POSTNATAL DEVELOPMENT OF PERIPHERAL CHEMOSENSITIVITY

Rachel Landauer
Department of Physiology, University of Birmingham, Birmingham B15 2TT

Peripheral chemoreceptors function as transducers of arterial blood gas tensions and pH and an homeostatic role for these receptors in the mediation of resting ventilation and exercise hyperpnoea has been forwarded on numerous occasions. We are interested in the mechanisms by which this receptor system adapts, or 'resets', to the natural changes in blood gas chemistry that occur and the consequence upon this development of an imposed chronic hypoxaemia.

Resetting in the *in vitro* carotid body preparation

Our initial objective was to establish an *in vitro* carotid body preparation to determine whether postnatal changes in peripheral chemoreceptor hypoxic sensitivity would occur in the absence of neural and humoral influences. Rat carotid bifurcations were excised under halothane-anaesthesia and superfused with warmed, bicarbonate-buffered saline solution. Extracellular recordings of afferent-fibre activity were recorded from the cut end of the carotid sinus nerve using glass suction electrodes while the Po_2, Pco_2 and temperature of the superfusate were continually monitored. Recordings of chemoreceptor discharge responses to ramp decreases in Po_2, at four fixed levels of Pco_2, in carotid bodies from adult (>35 days postnatal age) and neonatal (5–7 days) rats established that an increase in the sensitivity of these receptors to hypoxia did indeed occur *in vitro*[1] as had been previously reported to occur *in vivo*,[2] thus indicating resetting to be a process intrinsic to the developing carotid body. Our data also demonstrated that a greater than additive interaction between Po_2 and Pco_2 was present in the adult but absent in the neonate, i.e. that peripheral chemoreceptor sensitivity to CO_2 , while augmented in the adult by hypoxia, was unaffected by the level of Po_2 in the newborn.

Effect of chronic hypoxaemia from birth

The effect of chronic hypoxaemia from birth upon *in vitro* carotid body chemosensitivity was observed in rats > 35 days of age which had been born into and reared in an environmental chamber in which inspiratory oxygen was maintained at 12%. Using a similar protocol to that described above, we were able to demonstrate that a prevention of the natural postnatal elevation in arterial Po_2 could lead to a 'blunting' of hypoxic chemosensitivity, whereby the chemoreceptor discharge responses to hypoxia in chronically-hypoxic, adult animals were virtually indistinguishable from those of 5–7 day old, normoxic neonates.[3] Further, this failure to reset was coupled with a failure to develop a multiplicative interaction between CO_2 and hypoxia.

Conclusion

We suggest that the resetting of carotid body hypoxic chemosensitivity may be due to the development of a multiplicative interaction between CO_2 and hypoxia rather than a development to hypoxia *per se*. This process appears crucially dependent upon the level of arterial oxygenation and therefore could be greatly impaired by any neonatal cardiorespiratory disorders predisposing to chronic hypoxaemia. Currently, we are investigating the cellular mechanisms which might underlie this development.

References

1 Pepper, D R, Landauer, R C and Kumar, P. 1995. *J. Physiol.*, **485**, 2, 531–541.
2 Blanco, C E, Dawes, G S, Hanson, M A and McCooke, H B. 1984. *J. Physiol.*, **351**, 25–37.
3 Landauer, R C, Pepper, D R and Kumar, P. 1995. *J. Physiol.*, **485**, 2, 543–550.

LEVELS OF FREE TRIIODOTHYRONINE (FT3) AND FREE THYROXINE (FT4) IN PREMATURE INFANTS DURING THE FIRST THREE DAYS OF LIFE – RESULTS FROM THE PILOT STUDY OF THE THORN TRIAL (THYROID HORMONE REPLACEMENT IN NEONATES)

Sumita Biswas
Academic Child Health, Charing Cross and Westminster Medical School, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH

The THORN TRIAL is a planned multicentre randomized double blind placebo controlled clinical trial of Triiodothyronine (T3) and Cortisol given in replacement doses to preterm infants less than 30 weeks gestation. The purpose of this trial is to establish whether this treatment is able to reduce the incidence of lung disease and thus increase survival. As a prelude to this trial, a pilot study is in progress to determine the dose of T3, which will ensure that the plasma levels of this hormone are close to that normally seen in term infants.

We measured free T3 (FT3) and free T4 (FT4) levels in three groups of preterm infants (less than 30 weeks gestation) within 5 hours of age and subsequently at 24, 48, and 72 hours of age. Group I(n=10) did not receive any T3 (control group), group II (n=4) received 3 mcg/kg/day T3 as a continuous intravenous infusion for an average of 4 days, and group III (n=6) received intravenous T3 at a dose of 5 mcg/kg/day continuously for 7 days.

Results

Table 1: FT3 levels – pmol/1

	group I (control)		group II (3 mcg/kg/day T3)			group III (5 mcg/kg/day T3)		
Age	Mean	SEM	Mean	SEM	P*	Mean	SEM	P*
<5 h	4.38	0.56	5.65	1.48	NS	4.25	0.82	NS
24 h	4.27	0.51	4.85	0.32	NS	8.90	0.49	**0.001**
48 h	3.64	0.42	4.45	0.75	NS	6.97	0.98	**0.01**
72 h	3.85	0.83	4.60	0.83	NS	6.25	1.32	NS

*Mann–Whitney U $p<0.05$ NS=not significant
The FT3 levels in groups II and III at < 5 hours of age were taken before T3 infusion started.

Table 2: Mean FT4 levels – pmol/1

	group I (control)		group II (3 mcg/kg/day T3)			group III (5 mcg/kg/day T3)		
Age	Mean	SEM	Mean	SEM	P*	Mean	SEM	P*
<5 h	15.8	1.81	16.2	3.77	NS	12.0	2.45	NS
24 h	13.0	3.59	8.4	1.32	**0.04**	10.0	2.45	NS
48 h	10.6	122.0	6.8	1.27	NS	8.2	1.32	NS
72 h	9.5	1.2	6.9	2.29	NS	7.0	1.14	NS

*Mann–Whitney U $p<0.05$ NS=not significant
The FT4 levels in groups II and III at < 5 hours of age were taken before T3 infusion started.

Published data indicates that FT3 levels in term infants peak (to about 1200 pg/100ml or 18 pmol/l) at 24 hours of age and then declines gradually thereafter. Our study suggests that in preterm infants FT3 levels remain low throughout the first 3 days of life. We were not able to raise FT3 levels significantly with 3 mcg/kg/day T3 but obtained levels closer to that seen in term infants by increasing the dose to 5 mcg/kg/day. A possible reason for needing a higher dose than anticipated may be due to rapid metabolism of FT3 in preterm infants. Our results also show that there is a gradual decay of FT4 levels in the first 3 days of life. There was no statistical difference ($p>0.05$, Mann–Whitney U) in the mean FT4 level at 72 hours between the three groups. This would suggest, therefore, that the administration of T3 does not lead to significant suppression of FT4.

References

1 Erenberg, A, Phelps, D L, Lam, R and Fisher, D A. 1974. *Pediatrics*, **53**(2), 211

2 Eggermont, E, Vanderschueren Loderwyckx, M, De Nayer, P, Smeets, E, Vanacker, Y, Cornette, C, Jaeker, J, Devlieger, H, Eeckels, R and Beckers, C. 1984. *Helv. paediatr. acta.*, **39**(3), 209–222

THE EFFECTS OF CATIONS ON A CHLORIDE-DEPENDENT PHOSPHORYLATION SYSTEM IN HUMAN RESPIRATORY EPITHELIUM *IN VITRO*

L J Marshall and A Mehta
Department of Child Health, Ninewells Hospital and Medical School, Dundee DD1 9SY

The intracellular concentration of chloride ($[Cl^-]_i$) is an important regulatory signal in epithelial ion transport. Both $[Cl^-]_i$ and phosphorylation are known to affect the activity of apical ion channels,[1] such as the epithelial apical Cl^- channel CFTR (cystic fibrosis transmembrane conductance regulator), which is activated by phosphorylation.[2] Cystic fibrosis (CF) is the most common lethal genetic disease of Caucasians and is caused by mutations in the gene encoding CFTR with subsequent misprocessing and/or malfunction of the channel. The mechanism by which dysregulation of Cl^- transport generates CF pathophysiology is not fully understood. CF airway epithelia also have an abnormally high rate of Na^+ absorption, due to increased apical Na^+ permeability. Recent evidence shows that normal CFTR can also function as a negative regulator of epithelial Na^+ channels[3] and may regulate a volume-activated K^+ channel in intestinal crypts.[4] $[Cl^-]_i$ is of major importance in epithelial cell volume regulation and it has been proposed that it is the $[Cl^-]_i$ that is 'sensed', rather than the volume of the cell.[5] The existence of a Cl^--dependent, non-specific cation channel has recently been proposed to provide a mechanism regulating Cl^- secretion and Na^+ reabsorption following osmotic stress.[6] We have previously reported that an apically enriched fraction of human nasal respiratory epithelium contains at least two endogenously active protein kinases which phosphorylate their target proteins in a chloride-dependent and anion-dependent manner.[7] One kinase utilizes GTP as phosphate donor to phosphorylate a 37kDa protein (p37) whereas the other uses ATP to phosphorylate p45 – a 45 kDa protein. Recently we have studied the cation dependency of the phosphorylation of p37 using the chloride salts of sodium, potassium and ammonium. The results show that these cations regulate the intensity of phosphorylation of p37 in a dose-dependent manner, which differs from the chloride-dependent phosphorylation of these proteins. The greatest decrease in phosphorylation is seen with the ATP kinase at physiological $[Na^+]_i$ (10mM). This may reflect the physiological response of membrane phosphoproteins to increased concentrations of cations and chloride. Our new data suggests that the apical membrane protein structure is dependent on the concentrations of sodium and chloride ions.

References

1 Wang, X, Marunaka, Y, Dho, S, Foskett, J K and O'Brodovich, H. 1993. *Can. J. Physiol. Pharmacol.*, **71**, 645–649
2 Bear, C E, Li, C, Kartner, N, Bridges, R J, Jensen, T J, Ramjeesingh, M and Riordan, JR. 1992. *Cell*, **68**, 809–818.
3 Stutts, M J, Canessa, C M, Olsen, J C, Hamrick, M, Cohn, J A, Rossier, B C, Boucher, R C. 1995. *Science*, **269**, 847–850.
4 Valverde, M A, O'Brien, J A, Sepulveda, F V, Ratcliff, R A, Evans, M J and Colledge, W H. 1995. *PNAS*, **92**.
5 Foskett, J K. 1995. *In press.* Proc. Phys. Soc., Cork
6 Chan, H C, and Nelson, D J. 1992. *Science*, **257**, 669–671.
7 Treharne, K J, Marshall, L J and Mehta, A. 1994. *Am. J. Physiol.*, **257**, L592–L601.

THE ROLE OF MACROPHAGES IN INFLAMMATORY LUNG DISEASE

Shelley Folkard
The Lung Research Group, Department of Medicine, Medical School Unit, Southmead Hospital, Westbury on Trym, Bristol BS10 5NB

We are interested in the role of the alveolar macrophage in determining the outcome of the inflammatory response within the lung. All of our work is aimed at determining those factors which cause this important part of the host defence mechanism to become excessive, inappropriate or uncontrolled, leading to tissue damage. We have studied inflammatory mechanisms in several diseases including the adult respiratory distress syndrome (ARDS), sarcoidosis and cryptogenic fibrosing alveolitis (CFA), concentrating on the production and activity of proximal mediators such as tumour necrosis factor (TNF). More recently, we have looked at other aspects of macrophage function, namely their potential role as coordinators of inflammation in asthma by their production of chemokines. We have shown that cultured macrophages from allergic and non-allergic asthmatic subjects have an increased production of the chemokines IL-8 and MCP-1, but that chemokine RANTES is increased only in the case of non-allergic asthma. Furthermore, using a Boyden chamber assay and APAAP techniques, we have demonstrated an increased sensitivity of peripheral $CD8^+$ T cells from non-allergic asthmatics to RANTES *in vitro*. Other reports have described an influx of $CD8^+$ cells into the airways of non-allergic asthmatics. Our data suggests that the production of RANTES by macrophages is one plausible mechanism by which $CD8^+$ T cells could migrate from the periphery into the lung, and supports our hypothesis of the macrophage as a major player in the control of inflammatory lung conditions.

CYTOKINE INDUCTION IN PULMONARY CD4$^+$ AND CD8$^+$ T-CELLS DURING INFECTION WITH RESPIRATORY SYNCYTIAL VIRUS

Tracy Hussell
Respiratory Medicine, Imperial College of Science, Technology and Medicine, Norfolk Place, Paddington, London W2 1PG

T-cell mediated lung inflammation is one of the key areas of research in pulmonary medicine. Many poorly understood lung disorders (including sarcoidosis, cryptogenic fibrosing alveolitis, extrinsic allergic alveolitis and asthma) are characterized by local infiltration with activated T-cells. The causes of T-cell activation in these diseases are, at best, only partially understood. Our studies of the roles of different T-cell subsets in the disease caused by a well characterised human common cold virus, respiratory syncytial virus (RSV), provide a framework for understanding some aspects of these disorders.

Viral bronchiolitis is the commonest single cause of infantile hospitalization in the Western world, and most cases are caused by RSV. Not only does RSV cause considerable morbidity and some mortality in children, but there are well established associations between bronchiolitis and the later development of recurrent wheezing (and sometime asthma) in later childhood. There is also an association with atopic disease, the nature of which is controversial. Our work has shown that RSV bronchiolitis in mice is largely caused by T-cell immunity to the virus.[1,2]

Helper T-cells can be classified functionally into two main types: Th1 cells play a major role in eliminating viral pathogens, while Th2 cells mediate anti-parasite immunity but may also mediate allergic responses. These functions are thought to depend on characteristic and distinct patterns of cytokine production, but a deeper understanding of the relevance of these subsets to disease has been difficult to obtain using previously available techniques. We, therefore, refined flow cytometric methods to study intracellular cytokine production within T-cells.[3] Using this method, we have observed the true complexity of cytokine production in T-cells during normal inflammatory responses to viral infection on a cell-for-cell basis. We compared the results of reverse transcriptase-PCR of cellular cytokine mRNA and flow cytometric analysis of intracellular cytokine production on bronchoalveolar lavage and mediastinal lymph node cells before and after intranasal infection with RSV. An early IFN-γ response occurred at both sites, but resolved quickly in the lymph nodes. Some early IFN-γ^+ also expressed IL-10. Only low levels of IL-2, IL-4 and IL-5 mRNA or protein expression could be detected at any time at either site. Even under conditions known to produce intense lung eosinophila.[4] IFN-γ producing cells still predominate but the proportion and intensity of IFN-γ expression is modulated. Co-expression of IFN-γ and IL-4 has not, so far, been seen in any sample of cells.

The RSV model has, therefore, proved fruitful for the examination of many important imunological questions in a biologically complex setting, and was the first viral infection in which it was possible to show the relevance of Th1/2 subdivisions. We are now exploring how established Th1/2 responses can be switched, a question of utmost importance to the treatment of chronic diseases characterized by polar cytokine production.

References

1 Openshaw, P J M. 1995. in 'Viral Immunopathology', R. Zinkernagel, (ed) Spinger Seminars in Immunopathology.

2 Alwan, W H, Kozlowska W and Openshaw, P J M. 1994. *J. Exp.Med.*, **179**, 81–87.

3 Openshaw, P J M, Murphy E E, Hosken N A, Manio V, Davis K and O'Garra A. 1995. *J.Exp.Med.*, in press, November 1995.

4 Openshaw, P J M, Clarke, S L and Record, F M. 1992. *Internat. Immunol.*, **4**, 493–500.

ION CHANNELS AND THEIR POSSIBLE ROLES IN HYPOXIC CHEMOTRANSDUCTION BY TYPE I CELLS OF THE RAT CAROTID BODY

Christopher J Hatton, Elisabeth Carpenter and Chris Peers
Institute for Cardiovascular Research, University of Leeds, Leeds LS2 9JT

Neurotransmitter-releasing type I cells are accepted as the O_2-sensing elements of the carotid body, and there is growing evidence to suggest that plasma membrane ion channels of type I cells are important in the mechanisms underlying the responses of these cells to hypoxia.[1,2] For this reason, we isolate type I cells enzymatically from rat carotid bodies, and investigate their ion channels using patch-clamp techniques. To date, we have identified two K^+ currents (termed IK_{Ca} and IK_v) and inward Ca^{2+} currents (which are partially attributable to L-type, dihydropyridine-sensitive Ca^{2+} channels)[2]. Hypoxia (pO_2 *c.* 20 mmHg) inhibits IK_{Ca} in type I cells,[3,4] which results in cell depolarization, activation of Ca^{2+} channels and hence, presumably, neurosecretion. We believe that inhibition of IK_{Ca} is not a membrane-limited effect, but requires cytosolic factors.[4] Our current studies are aimed at determining these factors, and our recent findings suggest that there may be an involvement of cytochrome P-450: pharmacological P-450 inhibitors appear to attenuate the inhibition of K^+ currents by hypoxia in type I cells.[5]

We are also investigating the ion channels of type I cells from rats born and reared in chronic hypoxia (10% O_2). These animals do not respond to acute inspired hypoxia with an elevated ventilation (unlike normoxically-reared rats), and their carotid bodies are enlarged. We have shown that type I cells from these rats appear to lack IK_{Ca}, but the remaining IK_v is reversibly inhibited by hypoxia.[6] However, the cells do not depolarize in response to hypoxia, presumably because IK_v is not active at the cell resting membrane potential.

Our current and future studies are aimed at elucidating more fully the mechanisms underlying acute hypoxic inhibition of K^+ channels in normoxic and chronically hypoxic type I cells, and how these effects are reflected in the ultimate output of the type I cell, which is neurotransmitter release. We shall also be investigating whether ion channels are regulated by autoreceptors for the numerous transmitter substances released by the type I cells.

References

1 Gonzalez, C, Almaraz, L, Obeso, A and Rigual, R. 1994. *Physiol. Rev.*, **74**, 829–898.
2 Peers, C, and Buckler, K J. 1995. *J. Memb. Biol.*, **144**, 1–9.
3 Peers, C. 1991. *Neurosci. Lett.*, **119**, 253–256.
4 Wyatt, C N and Peers, C. 1995. *J. Physiol.*, **483**, 559–565.
5 Hatton, C J and Peers, C. 1995. (submitted to Physiol. Soc. Meeting, King's College, Dec. 1995)
6 Wyatt, C N, Wright, C, Bee, D and Peers, C. 1995. *Proc. Natl. Acad. Sci., USA*, **92**, 295–299.

THE EFFECTS OF BRONCHIAL MYOFIBROBLASTS ON HUMAN EOSINOPHIL SURVIVAL *IN VITRO*

William Roche and Shaoli Zhang
Pathology Department, University of Southampton, South Block, Level E, Southampton General Hospital, Southampton SO16 6YD

Tissue eosinophilia is a characteristic of allergic inflammation such as asthma. The mechanisms for the eosinophil accumulation and activation are crucial for the pathogenesis of bronchial asthma. The specialized fibroblasts, myofibroblasts, beneath the bronchial basement membrane may have important role in this process by their proximity to infiltrating eosinophils and their cytokine and extracellular matrix production. In this study we have shown that eosinophil survival was significantly increased (92%) by co-culture with myofibroblasts compared to cultured alone (2%). Conditioned medium from TNFα stimulated myofibroblasts also significantly prolonged eosinophil survival (61.3%) and this effect could be blocked by GM-CSF antibody. GM-CSF secretion by myofibroblasts was induced in co-culture and also by eosinophil-conditioned medium. The survival enhancing activity and GM-CSF induction in the co-cultures could be inhibited by neutralizing antibodies against both TNFα and IL-1α, and also by prednisolone. It suggested that both cell/cell contact and soluble eosinophil-derived cytokines are involved in the interaction of eosinophils with myofibroblasts resulting in a TNFα/IL-1 mediated release of GM-CSF from myofibroblasts which inhibits eosinophil apoptosis.

A MODEL OF A MAMMALIAN LUNG ALVEOLAR DUCT

Edgar Denny
Physiological Flow Studies Group, Centre for Biological and Medical Systems, Imperial College of Science, Technology and Medicine, London SW7 2BX

The alveolar duct is the smallest functional unit within the mammalian lung parenchyma. The elastin and collagen connective tissue network, together with the surface tension forces, are the principal loadbearing components. Thick fibre bundles of elastin and collagen bear the forces in the alveolar mouths forming the duct while smaller fibre bundles together with the surface tension of the air–liquid interfaces bear the forces in the surrounding alveolar septa.[1] The micromechanical forces that tend to pull the alveolar mouths radially inward are balanced by opposing forces in the alveoli that tend to retract the duct radially outward. Diseases such as emphysema and fibrosing alveolitis are often associated with a remodelling of connective tissue caused by changes in the distribution of tissue or in the size of the airspaces.[2] The effect of such changes upon the micromechanical behaviour of parenchyma is not well understood. At the microscopic structural level, a scale at which experimental investigation is difficult, modelling can provide a useful role in investigating alveolar duct functional properties. A model of the mechanical properties of an alveolar duct is analysed using a finite element method.[3] The geometry of each alveolus is modelled using a truncated octahedron; a polyhedron with six square and eight hexagonal faces. The duct is modelled by an assemblage of thirty-six polyhedra arranged to represent a longitudinal airspace surrounded by alveoli. Pin-jointed line elements model the elastin and collagen fibre bundles around the alveolar mouths and across the alveolar septa. Separate stress – strain laws define the characteristics of the elastin and collagen fibre bundles and an area dependent relationship defines the surface tension characteristics of the air–liquid interfaces. The duct dimensions and connective tissue distributions are based on published data for humans.[1] The model is used to investigate how changes in the microstructural properties influence the alveolar duct pressure–volume (PV) behaviour. The effects of varying the alveolar dimensions, the total volume density of connective tissue, and the distribution of connective tissue between the alveolar mouths and septa are studied. Static PV curves are computed for the model that compare well with experimental data.[3] Distensibility indices *(K)* are calculated from the deflation PV curves to characterize the effects of the modifications. The ratio of total duct airspace volume to total alveolar volume is computed over the PV loop to examine the degree of geometric anisotropy. It was found that the air filled lung distensibility K_a fell with a reduction in the alveolar airspace size but increased with a reduction of total connective tissue volume density. Experimental studies have found a similar relationship between K_a and the mean intercept length in emphysematous lungs.[2] The saline filled lung distensibility K_s remained constant with alveolar size and increased with decreasing total connective tissue volume density. To achieve near isotropic expansion, models with smaller alveolar dimensions required a greater proportion of their total connective tissue in the alveolar mouths. Interspecies comparisons have found a similar relation between tissue distribution and alveolar dimensions.[4]

References

1 Mercer, R R and Crapo, J D. 1990. *J. Appl. Physiol.*, **69**, 756–765.
2 Greaves, I A and Colebatch, H J H. 1980. *Am. Rev. Respir. Dis.*, **121**, 127–136.
3 Denny, E and Schroter, R C. 1995. Trans. *ASME J. Biomech. Eng.*, **117**, 254–261.
4 Mercer, R R, Russell, M L and Crapo, J D. 1994. *J. Appl. Physiol.*, **62**, 1480–1487.

ION CHANNELS IN FRESHLY ISOLATED AND CULTURED HUMAN BRONCHIAL SMOOTH MUSCLE CELLS

Vladimir Snetkov
Department of Allergy and Respiratory Medicine, UMDS, Lambeth Palace Road, London SE1 7EH

Smooth muscle cells isolated from tissue and grown in cell culture undergo considerable morphological and biochemical transformation, which is generally described as a transition from a contractile to a non-contractile (proliferative or synthetic) phenotype. While it is not clear how well airway smooth muscle cells growing in culture mimic airway wall remodelling during chronic severe asthma, cell culture models represent the only controllable and accessible means available for the study of proliferation and many molecular biological processes. There have, however, been few studies concerning the changes in electrophysiological properties of smooth muscle as the phenotype changes from a contractile to a proliferative type, although such changes may have profound effects on function.

We have studied voltage-gated ion currents in human bronchial airway smooth muscle cells. Two different groups of cells in culture (proliferating or growth-arrested) were compared with freshly isolated (native contractile) cells.

All three groups of human bronchial cells were found to possess numerous large conductance charybdotoxin-sensitive potassium channels. However, there was evidence that changes in cell phenotype lead to the expression of channels with different properties. Three major types of channels with conductances of 206±30 pS (n = 30), 144±11 pS (n = 26) and 109±5 pS (n = 14) have been found in all groups (inside-out patches, symm. 140 mM-KCl, $[Ca^{2+}]_i$ < 0.1 nM). In the patches from *freshly isolated cells* large conductance potassium channels were represented mainly with 206 pS conductance. As we reported earlier,[1] these channels were active at physiological membrane potential even at very low free Ca^{2+} concentration. Intracellular GTP significantly increased open state probability. In a few patches 144 pS and 109 pS channels were also found. In the membrane of *proliferating cells* in culture in the presence of 10% fetal calf serum channels of the same conductance could be observed under similar conditions; however, most channels had the conductances of 144 pS and 109 pS. At low $[Ca^{2+}]_i$ used in our experiments 144 pS channels could be recorded only at very depolarized potentials and were not sensitive to intracellular GTP, while 108 pS channels were found to be active at wide range of membrane potentials, but only in the presence of GTP on the cytosolic site of membrane. Corresponding difference in outward whole-cell currents between freshly isolated cells and those growing in culture was also observed. *Growth-arrested cells* kept without serum 24–96 h revealed the same set of channels as the proliferating cells. However, the relative frequency of GTP-dependent 109 pS channels was much higher.

In a proportion of proliferating cultured cells a tetrodotoxin-sensitive Na^+ current and a hyperpolarization-induced inwardly rectifying K^+ current were also observed. Both the occurrence and amplitude of these currents in growth-arrested cells were substantially diminished.

The changes in ionic conductances found in the proliferating cultured cells would tend to make the tissue more excitable, and it is possible that this could underlie the increased excitability found in asthmatic airways, when smooth muscle proliferation is known to occur.

Reference

1 Snetkov, V A, Hirst, S J, Twort, C H C and Ward, J P T. 1995. *Br. J. Pharmacol.*, **115**, 1117–1125.

EFFECTS OF CHOLINERGIC AGONISTS ON INTRACELLULAR CALCIUM IN RAT CAROTID BODY TYPE I CELLS

Leonardo L T Dasso, Keith J Buckler and Richard D Vaughan-Jones
University Laboratory of Physiology, Parks Road, Oxford OX1 3PT

The carotid body is a sensor of arterial PO_2, PCO_2 and pH. The type I cell is the primary chemosensory element within the carotid body. Transduction of physiological stimuli in the type I cell is currently thought to involve a rapid rise in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through activation of voltage-operated Ca^{2+} channels, which promotes secretion of neurotransmitters, e.g. dopamine,[1] increasing carotid sinus nerve discharge to the central respiratory centre. While $[Ca^{2+}]_i$ responses to changes in PO_2, PCO_2 and pH have been extensively investigated in the type I cell, no detailed studies have been concerned with receptor-mediated $[Ca^{2+}]_i$ changes.[2,3]

In the carotid body acetylcholine is present exclusively in the type I cells and is released during carotid body stimulation. The discharge frequency of the carotid nerve is extremely sensitive to exogenous acetylcholine. Furthermore, the nicotinic antagonist, α-bungarotoxin, significantly decreases hypoxia-induced release of dopamine and chemoreceptor activity from cat carotid bodies, suggesting that activation of acetylcholine receptors and dopamine release are, at least, partially coupled.[2]

We have studied the effects of cholinergic agonists upon $[Ca^{2+}]_i$ in enzymically isolated neonatal rat single type I cells using fluorescence microscopy.[4] Acetylcholine elevated $[Ca^{2+}]_i$; maximal increases were observed with 100 μM-ACh (798±76 nM above basal; means ± SEM, n = 89). Muscarinic agonists (muscarine, methacholine, oxotremorine and oxotremorine M) also elevated $[Ca^{2+}]_i$; maximal $[Ca^{2+}]_i$ rises were evoked by 300 μM-methacholine (672±87 nM over basal; n = 39). Finally, nicotine or the nicotinic agonist dimethylphenylpiperazinium elevated $[Ca^{2+}]_i$; maximal increases of $[Ca^{2+}]_i$ (701± 80 nM, n = 42) were observed with 100 μM-nicotine. Muscarinic and nicotinic responses were prevented by 1 μM-atropine or 1 μM-mecamylamine, respectively. Removal of extracellular Ca^{2+} (1mM-EGTA) rapidly abolished nicotinic responses, while activation of muscarinic receptors led to transient elevations of $[Ca^{2+}]_i$ even after several minutes in Ca^{2+}-free medium. In addition to stimulating the release of Ca^{2+} from intracellular stores, muscarinic agonists activated Ca^{2+} influx, as evidenced by Mn^{2+} quench of intracellular Fura-2 signals.[5]

We conclude that both nicotinic and muscarinic acetylcholine receptors influence $[Ca^{2+}]_i$ but rely on different sources of Ca^{2+} to do so. Nicotinic responses depend on Ca^{2+} entry from the extracellular medium. Muscarinic receptors evoke Ca^{2+} release from intracellular stores, which is followed or accompanied by Ca^{2+} influx. Our data suggest that acetylcholine may activate autoreceptors on type I cells to evoke changes in $[Ca^{2+}]_i$ and consequently modulate neurotransmitter release.

References

1 Ureña, J, Fernandez-Chacon, R, Benot, A R, Alvarez de Toledo, G A and Lopez-Barneo, J (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 10208–10211.
2 Fidone, S J and González, C (1986) *The Respiratory System, Handbook of Physiology* (eds. Cherniak, N S and Widdicombe, J G) Sect. 3, Vol. II Chapter 9, 247–312.
3 Peers, C and Buckler, K J. 1995 *J. Membrane Biol.*, **144**, 1–9.
4 Buckler, K J and Vaughan-Jones, R D. 1993 *Pflügers Arch.*, **425**, 2227.
5 Merritt, J E, Jacob, R, Hallam, T J. 1989 *J. Biol. Chem.*, **264**, 1522–1527.

INTRACELLULAR CYCLIC NUCLEOTIDES MEDIATE AND REGULATE LIQUID ABSORPTION IN THE POSTNATAL LUNG

Richard Stephens
Dept of Child Health, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE

The air-filled mammalian lung has the capacity to absorb liquid across its epithelium. This capacity persists throughout postnatal life, beginning at birth when the process is vital to the adaptation to normal air-breathing. The central thrust of our research has been to unravel the intracellular mechanisms which regulate and/or mediate this process. Much of our work has centred around the role of intracellular cyclic nucleotides (cNuc), such as cAMP and cGMP, which we have studied by inhibiting the degradation of cNuc by cNuc phosphodiesterase (PDE) isoenzymes. Experiments were done in postnatal sheep perfused *in situ*[1] which were filled with artificial lung liquid (LL) containing an impermeant tracer which allowed measurement of LL absorption rate.

Theophylline, a nonselective PDE inhibitor that blocks the degradation of several cNuc, increased LL absorption rate with respect to resting rate in neonatal sheep aged 0–14 days and juvenile sheep aged 6–12 weeks. This effect was blocked by the ß-blocker sotalol in lungs from neonatal but not juvenile, sheep.[2] This suggested that a component of lung liquid absorption was mediated by cNuc, and that in neonates, this component was mediated entirely by cAMP originating from -adrenergic stimulation. The data also suggested that the cNuc-dependent component of LL absorption in juvenile sheep was mediated either by cAMP from some source other than ß-adrenergic stimulation or by cNuc other than cAMP, e.g. cGMP. Zaprinast, a selective inhibitor of the PDE isoenzyme specific for cGMP, was found to have no effect on LL absorption rate.[4] This eliminated the possibility that cGMP accounted for the ß-blocker-insensitive, cNuc-dependant component of LL absorption in juvenile lungs. Rolipram, a selective inhibitor of cAMP-specific PDE, increased LL absorption rate in neonatal and juvenile lungs.[3] Sotalol has been found to block this effect in neonates but not in juveniles, which confirmed that the ß-blocker-insensitive, cNuc-dependant component of LL absorption in juvenile lungs is mediated by cAMP from sources other than ß-adrenergic stimulation.

The possibility that another cNuc, cCMP, is involved in mediating LL absorption cannot yet be ruled out, however, since there is a component of LL absorption at rest which is both ß-blocker and theophylline-insensitive and 'conventional' PDE inhibitors, such as theophylline and rolipram, to not block cCMP hydrolysis.[5] Similarly, the effects on LL absorption of 'minor' cNuc such as cUMP, cdTMP and cIMP remain unknown.

References

1 Ramsden C A, Markiewicz M, Walters D V, Gabella G, Parker K A, Barker P M and Neil H L. 1992. *Journal of Physiology*. **448**, 579–597
2 Stephens R H, Benjamin A R and Walters D V. 1993. *Journal of Physiology*. **459**, 333
3 Stephens R H, Benjamin A R and Walters D V. 1993. *Journal of Physiology*. **467**, 341
4 Stephens R H, Benjamin A R and Walters D V. 1995. *Journal of Physiology*. In Press
5 Helfman D M and Kuo J F. 1982. *Biochem. Pharmacol.*, **31** 43–47

MECHANISMS UNDERLYING HYPOXIC PULMONARY VASOCONSTRICTION

Tom Robertson
Department of Allergy and Respiratory Medicine, UMDS, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH

The precise mechanisms underlying hypoxic pulmonary vasoconstriction (HPV) are still elusive. The recent discovery of K^+ channels that are depressed by hypoxia in pulmonary vascular smooth muscle has provided a potential signal transduction mechanism for linking a reduction in PO_2 to Ca^{2+} entry, but there are many reports suggesting that sustained HPV depends on the presence of the endothelium. Many endothelium-derived vasoactive factors have been investigated as possible mediators of HPV, yet none have been found to be indispensable for HPV.

We have been studying the mechanisms of HPV in small intrapulmonary arteries of the rat, and in common with other groups have found a complex biphasic relationship.[1] Upon induction of hypoxia a large, and transient, rise in tension was observed which reached a peak within 2–4 minutes (phase 1). The tension then fell over a period of 10–15 minutes, to a level above the pre-hypoxic tone, and this was subsequently followed by a slowly-developing contraction which reached a plateau after approximately 40–45 minutes (phase 2). Phase 1 was shown to be associated with a transient rise in $[Ca^{2+}]_i$. However, there was a dissociation between tension and $[Ca^{2+}]_i$ during phase 2 as, although tension increased, $[Ca^{2+}]_i$ was maintained at a level above that observed prior to hypoxia. This apparent sensitization of the contractile machinery to calcium was found to be protein kinase C, and also pH, independent.[2] The slowly developing, but sustained, phase 2 constriction was entirely dependent on the presence of an intact endothelium, suggesting the release of a vasoconstrictor or inhibition of release of a vasodilator. In the light of previous work the latter is unlikely, and all endogenous vasoconstrictors that have been investigated so far have proved to be not involved. We are, therefore, currently investigating whether any potential mediator is produced by the lungs during hypoxia. Isolated salt-perfused lungs were made hypoxic to various degrees, and the perfusate collected, concentrated, and partially purified. One of the extracts so obtained was found to constrict isolated rat IPA, but not systemic arteries. It was also shown to inhibit a delayed rectifier potassium current in isolated rat pulmonary artery smooth muscle cells. The active component of this extract has a molecular weight of less than 3000, and is heat stable (97°C, 20 minutes). We are now using HPLC and mass spectroscopy techniques in an attempt to purify further, and hopefully identify, this factor.

References

1 Leach, R M, Robertson, T P, Twort, C H and Ward, J P T. 1994. *American Journal of Physiology*, **266**, L223–L231.
2 Robertson, T P, Aaronson, P I and Ward, J P. 1995. *American Journal of Physiology*, **37**, H301–H307.

A MODEL OF TRACHEAL DRUG UPTAKE: EPITHELIAL DAMAGE ALTERS THE EFFECTS OF LUMINAL DRUGS ON TRACHEAL BLOOD FLOW AND TRACER PERMEABILITY IN ANAESTHETIZED SHEEP

Ursula Wells

Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, London SW17 ORE

Many drugs used to treat airway disease are taken by inhalation, yet little is known about the physiological and pathophysiological factors that affect their uptake across the airway wall. In our model[1] the uptake from tracheal lumen to venous blood of two inert tracers, with molecular weights similar to commonly used respiratory drugs, is measured. ^{99m}Tc-DTPA (MW 492 Da) is hydrophilic, so crosses the tracheal epithelium via paracellular pathways; ^{14}C-AP (^{14}C-antipyrine, MW 188 Da) is lipophilic and crosses transcellularly. Uptake of ^{99m}Tc-DTPA into venous blood is negatively related to blood flow.[1] Epithelial damage, common in asthma, could alter drug uptake from the airway and also expose sensory nerve endings. We have studied (i) the effects of tracheal epithelial damage on the permeability of both tracers and (ii) whether epithelial damage increases the effects of drugs given into the tracheal lumen on tracheal blood flow and tracer uptake. The drugs used were histamine (hydrophilic), which is often inhaled to test airway responsiveness, and capsaicin (lipophilic) which stimulates afferent nerves. Sheep were anaesthetized with sodium pentobarbitone (20 mg.kg^{-1} i.v.) and artifically ventilated.[1] Tracheal arterial flow was measured with an electromagnetic flow probe. A tracheal vein was cannulated, and blood collected for 5 min periods. A segment of trachea, isolated in situ, was filled with Krebs–Henseleit solution containing ^{99m}Tc-DTPA for 15 min periods or ^{14}C-AP for up to 90 min. The effect of epithelial damage (caused by 10 mM-H_2O_2) on baseline permeability to both tracers was measured. In a second series of experiments, 100 μM-histamine was washed into the lumen for 15 min before (Hist 1) and after (Hist 2) epithelial damage caused by 0.2% Triton X-100. In the final study, 10 μM-capsaicin was given before (Caps 1) and after (Caps 2) epithelial damage induced by H_2O_2. H_2O_2 increased the baseline permeability coefficient for ^{99m}Tc-DTPA (from $-2.6\pm0.8 \times 10^{-7}$ cm.s^{-1} to $-89.7\pm25.6 \times 10^{-7}$ cm.s^{-1}) but not for ^{14}C-AP ($-3312.6\pm878.2 \times 10^{-7}$ cm.s^{-1} before and $-2565.0\pm246.1 \times 10^{-7}$ cm.s^{-1} after damage). Epithelial damage significantly enhanced the blood flow response to both histamine and capsaicin [arterial flow, 0–5 min: Hist 1 +6.4±0.8%, Hist 2 +36.7±12.2% (n=5, p<0.05 unpaired t-test); Caps 1 +11.5±2.1%, Caps 2 +31.0±6.0% (n=5, p<0.05)]. Venous ^{99m}Tc-DTPA concentration is negatively related to blood flow,[1] and after epithelial damage both histamine and capsaicin produced a significantly greater reduction in venous ^{99m}Tc-DTPA concentration (0–5 min: Hist 1 +5.2±7.0%, Hist 2 –37.7±6.9%; p<0.05; Caps 1 –7.3±6.9%, Caps 2 –34.9±4.7%, p<0.05). Thus, epithelial damage increases the uptake of hydrophilic but not lipophilic molecules. This may explain the greater effects after epithelial damage of histamine but not the enhanced effects of capsaicin. The increased response to capsaicin may be due to greater exposure of nerve endings, to loss of epithelial neutral endopeptidase or to a sensitization of the nerves to capsaicin by H_2O_2. Finally, the results suggest that luminal drugs that cause vasodilation will modulate their own uptake, and that this effect will be greater after epithelial damage.

Reference

1 Hanafi, Z, Corfield, D R, Webber, S E, and Widdicombe, J G. 1992. *J. Appl. Physiol.*, **73**, 1273–1281.

BACTERIAL INTERACTIONS WITH THE RESPIRATORY MUCOSA

Alan D Jackson and Robert Wilson
Host Defence Unit, Imperial College of Science, Technology and Medicine, National Heart and Lung Institute, Emmanuel Kaye Building, Manresa Road, London SW3 6LR

The interaction of infectious agents with the human respiratory mucosa has been studied using organ cultures, cell culture monolayers and dispersed cells. Organ cultures offer the advantage of intact tissue containing numerous differentiated cell types, but are usually unphysiological in that they need to be immersed in media. We have developed a human respiratory mucosa organ culture incorporating an air-interface which remains viable for 20 days. Ciliary beat frequency (CBF) fell slightly but significantly ($p<0.05$) from 11.6±0.2 Hz at time 0, to 10.6±0.3 Hz after 20 days. Scanning electron microscopy showed that the proportion of the organ culture surface covered by mucus, ciliated and non-ciliated cells did not change, and transmission electron microscopy showed no tight junction separation, mitochondrial abnormalities, or change in cilial density at 20 days compared to time 0.

We have used this model to study the interaction of isogenic *Streptococcus pneumoniae* variants, sufficient (PL+) and deficient (PL-) in the production of pneumolysin, with human respiratory mucosa. *S.pneumoniae* caused a progressive fall in CBF which became significant at 24 h for PL+ ($p<0.01$) but only at 48 h for PL- ($p<0.01$). Similarly, there was significant epithelial damage at 24 h for PL+ ($p<0.01$) but only at 48 h for PL- ($p<0.05$). PL+ alone caused separation of epithelial tight junctions and bacteria adhered to the edges of separated unciliated cells. This work implicates pneumolysin in creating a potential route for paracellular invasion of the respiratory epithelium by *S.pneumoniae*. Both variants in the present study adhered to the mucosa singularly or as diplococci. However, in immersed organ cultures *S.pneumoniae* adhered as chains of cocci, suggesting that the presence of medium may influence the behaviour of bacteria in the organ culture.

We have compared adherence of *Haemophilus influenzae* type b strain Eagan (Hib) to organ cultures with an air-interface or immersed in medium constructed from the same donor tissue. Infection caused significant epithelial damage ($p<0.05$), together with separation of tight junctions. When tissue was immersed bacterial adherence to the mucosa was sparse and non-discriminatory between mucus, damaged epithelium, ciliated cells or unciliated cells. In the air-interface organ culture, adherence was predominantly to mucus, but significant numbers also adhered to damaged epithelium and to the edges of separated epithelial cells. When there was severe cell separation bacteria were observed adherent to the underlying basal cells and basement membrane. Bacterial adherence was 55.8 times greater ($p<0.005$) to tissue maintained with an air-interface compared to immersed tissue, which suggests that immersion in culture medium affects the interaction of bacteria with the respiratory mucosa and that studies of such interactions should utilise air-interface organ cultures.

RESPIRATORY DISTRESS SYNDROME IN PRETERM INFANTS

Christine E Conner
Department of Obstetrics and Gynaecology, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY

Clinical perspective

Respiratory distress syndrome is the consequence of failure of lung development and in the UK 6000 preterm infants per annum suffer the severist form of the disease. Ventilatory support is successful for infants >30 weeks gestation, where respiratory distress is caused primarily by a deficiency of lung surfactant. Extreme preterm infants <30 weeks gestation, in addition, have inadequate differentiation of the respiratory portion of the lung and from 20 to 30 weeks gestation, the pattern of survival (0–98%) is directly related to that critical stage of lung development when terminal airsacs are being formed. In extreme preterm infants morbidity remains high and 20–30% of survivors will have long-term handicaps.

Research strategy

Our research strategy is to develop new and effective therapeutic methods to prevent respiratory distress syndrome in preterm infants through the understanding of the mechanisms of control of human fetal lung development.

Human lung development

During this critical stage of human lung development when terminal airsacs are being formed, the partially differentiated epithelium progresses through a series of intermediate cell types to the terminally differentiated state with the formation of mature type I (gas exchanging) and type II (surfactant producing-fluid transporting) pneumatocytes. The regulation of surfactant production by mature type II pneumatocytes has been extensively investigated but little is known of the regulation of the maturation of partially differentiated and intermediate cells to mature pneumatocytes, of critical importance to the survival of extreme preterm infants. Work in our laboratory indicates that prostaglandins have a key role in the regulation of this differentiation process[1] but there are presently no specific molecular markers for these important stages of maturation i.e. for partially differentiated and intermediate cell types, to allow us to further investigate the nature of these important processes.

Endoplasmic reticulum proteins and terminal differentiation

Prostaglandins are synthesized from arachidonic acid through the activity of prostaglandin H synthase, an enzyme which is located in the endoplasmic reticulum. We have shown that the expression of prostaglandin H synthase increases considerably around the time of epithelial differentiation both *in utero* and in self-differentiating lung organ culture. Other endoplasmic reticulum proteins and enzymes, which are normally found in adult liver, are also present in diverse range of developing human tissues.[2,3] For example, we have demonstrated that the glucose-6-phosphatase system[3] and UDP-glucuronosyltransferases are also expressed and functional in the developing human trachea and oesophagus using enzymic assays and monospecific molecular probes i.e. antibodies for immunoblotting and immunohistochemistry and cDNA probes for the detection of specific mRNA. More recent work indicates that the glucose-6-phosphatase system and UDP-glucuronosyltransferases increase with development and before terminal differentiation of this proximal epithelium and current investigations are delineating their expression in distal airway epithelium.

Conclusion

The role of the glucose-6-phosphatase system in developing fetal trachea is likely to be local and not for production of blood glucose as in the liver and that of UDP-glucuronosyltransferases for the detoxication of xeno- and endo-biotics with their use as molecular markers of epithelial differentiation a novel application.

References

1 Hume, R, Kelly, R, Cossar, D, Giles, M, Hallas, A, Gourlay, M and Bell, J. 1991. Self-differentiation of human fetal lung organ culture: the role of prostaglandins PGE2 and PGF2. *Exp. Cell Res.*, **194**, 111–117.

2 Hume, R, Voice, M, Pazouki, S, Giunti, R, Benedetti, A and Burchell, A. 1995. The human adrenal microsomal glucose-6-phosphatase system. *J. Clin. Endocrinol. Metab.*, **80**, 1960–1966.

3 Hume, R, Burchell, A, Allan, B B, Wolf, C R, Kelly, R, Hallas, A and Burchell, B. 1995. The ontogeny of key endoplasmic reticulum proteins in human embryonic and fetal red blood cells. *Blood*, (in press).

4 Hume, R and Burchell, A. 1995. The glucose-6-phosphatase enzyme in developing human trachea and oesophagus. *Histochemical J.*, (in press).

REGULATION OF SPECIFIC IMMUNE RESPONSES TO HOUSE DUST MITE DERIVED ALLERGENS

Gerard F Hoyne, Timothy Bourne and Jonathan R Lamb
Infection and Immunity Section, Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB

The basis of specific immunotherapy is to immunize susceptible individuals with allergen or derivatives under conditions that will inhibit allergen mediated-inflammatory responses. These responses are characterized by the production of specific IgE antibodies and inflammatory mediators released from infiltrating eosinophils, basophils and mast cells. The cytokines (IL-4, IL-5, IL-10 and IL-13) produced predominantly by $CD4^+$ (Th0 and Th2) cells are central in the regulation of allergic immune responses.[1] Because the activation of $CD4^+$ T cells requires recognition of peptide fragments of allergen bound to MHC class II molecules these cells are an obvious target of immunotherapy.[2]

From *in vitro* studies on human T cell clones we have observed that allergen derived peptides analogues can be presented under conditions that modify the function of specific Th0 and Th2 cells. Irrespective of their HLA class II restriction specificity, when exposed to supraimmunogenic doses of peptide, in the presence or absence of APCs, human $CD4^+$ T cells become refractory to an immunogenic challenge and fail to proliferate or provide B cell help, even though their responsiveness to IL-2 is enhanced. The loss of functional activity is accompanied by complex phenotypic changes and these include downregulation of TcR and the co-stimulatory receptor CD28, upregulation of CD25, and for some T cells comodulation of CD4 with TcR. During the induction of anergy cytokine specific mRNA levels are enhanced,[3] but anergic T cells when restimulated fail to secrete IL-4 and IL-5, although IFN-γ production remains unaltered (Th2 → Th1).[4,5]

To determine if peptides can modulate the function of HDM specific T cells *in vivo*, T cell recognition of a major allergen of house dust mite (Der p I) was investigated in $H\text{-}2^b$ mice. Following inhalation of peptide containing a dominant T cell epitope of Der p I transient T cell activation occurred prior to the inhibition of responses to both peptide and intact Der p I. T cells isolated from the tolerant mice, when restimulated *in vitro*, proliferated poorly, produced low levels of IL-2 and failed to provide cognate help for the production of Der p I specific antibodies.[6] In addition, peptide administered intranasally inhibited both ongoing and long-term immune responses to HDM derived allergens establishing that peptide mediated immunotherapy is effective in the regulation of established immune responses.

References

1 de Vries, J E. (ed). 1994. *Curr. Opin. Immunol.*, **7**, 835–873.
2 Hetzel, C and Lamb, J R. 1994. *Clin. Immunol. Immunopath.*, **73**, 1–10.
3 Schall, T J, O'Hehir, R E, Goeddel, D V and Lamb, J R. 1992. *J. Immunol.*, **148**, 381–387.
4 O'Hehir, R E, Yssel, H, Verma, S, de Vries, J E, Spits, H and Lamb, J R. 1991. *Int. Immunol.*, **3**, 819–826.
5 Yssel, H, Fasler, S, Lamb, J and de Vries, J E. 1994. *Curr. Opin. Immunol.*, **6**, 847–852.
6 Hoyne, G F, O'Hehir, R E, Wraith, D C, Thomas, W R and Lamb, J R. 1993. *J. Exp. Med.*, **178**, 1783–1788.

Delegates List

Dr Gareth Ackland
St John's College
University of Oxford
Oxford OX1 3JP

Dr Lewis Adams
Dept of Medicine
Charing Cross and Westminster Medical School
Fulham Palace Road
London W6 8RF

Professor Adrian Allen
Dept of Physiological Sciences
The Medical School
Framlington Place
Newcastle upon Tyne NE2 4HH

Dr Eric Alton
National Heart and Lung Institute
Emmanuel Kaye Building
Manresa Road
London SW3 6LR

Dr Christopher Baldwin
Dept of Immunology
The Medical School
Framlington Place
Newcastle upon Tyne NE2 4HH

Dr Denise Bee
Dept of Experimental Medicine
University of Sheffield Medical School
Beech Hill Road
Sheffield S10 2RX

Dr Maria Belvisi
Dept of Thoracic Medicine
National Heart and Lung Institute
Imperial College
Dovehouse Street
London SW3 6LY

Dr Sumita Biswas
Academic Child Health
Chelsea and Westminster Hospital
369 Fulham Road
London SW10 9NH

Dr Timothy Bourne
Dept of Biology
Infection and Immunity Section
Imperial College of Science, Technology and Medicine
Prince Consort Road
London SW7 2BB

Mr Tim Brazil
Dept of Veterinary Clinical Studies
Royal (Dick) School of Veterinary Studies
Veterinary Field Station
Easter Bush
Roslin
Midlothian EH25 9RG

Dr Keith Buckler
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Ms June Buffery
Academic Child Health
Chelsea and Westminster Hospital
369 Fulham Road
London SW10 9NH

Dr Anne Burke-Gaffney
Applied Pharmacology
National Heart and Lung Institute
Imperial College
Dovehouse Street
London SW3 6LY

Dr Jane Calvert
Dept of Immunology
The Medical School
Framlington Place
Newcastle upon Tyne NE2 4HH

Ms Elizabeth Carpenter
Institute for Cardiovascular Research
University of Leeds
Leeds LS2 9JJ

Dr Rachel Chambers
Centre for Cardiopulmonary Biochemistry and Respiratory Medicine
UCL Medical School
5 University Street
London WC1E 6JJ

Dr Edwin Chilvers
Dept of Medicine (RIE)
Respiratory Medicine Unit
Royal Infirmary
Edinburgh EH3 9YW

Ms Christine Clar
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr William Colledge
Dept of Physiology
University of Cambridge
Downing Site
Downing Street
Cambridge CB2 3EG

Dr Paul Collins
Dept of Applied Pharmacology
National Heart and Lung Institute
Dovehouse Street
London SW3 6LY

Dr Christine Connor
Dept of Obstetrics and Gynaecology
University of Dundee Medical School
Ninewells Hospital
Dundee DD1 9SY

Dr William Cookson
Nuffield Dept of Clinical Medicine
John Radcliffe Hospital
Headington
Oxford OX3 9DU

Dr Douglas Corfield
Dept of Medicine
Charing Cross and Westminster Medical School
Fulham Palace Road
London W6 8RF

Mr Mark Dagleish
Dept of Veterinary Clinical Studies
Royal (Dick) School of Veterinary Studies
Veterinary Field Station
Easter Bush
Roslin
Midlothian EH25 9RL

Dr Leonardo Dasso
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Edgar Denny
Centre for Biological and Medical Systems
Imperial College of Science, Technology
and Medicine
London SW7 2BX

Dr Carol Dezateux
Dept of Epidemiology and Biostatistics
Institute of Child Health
30 Guilford Street
London WC1N 1EH

Dr Paddy Dixon
Dept of Veterinary Clinical Studies
Royal (Dick) School of Veterinary Studies
Veterinary Field Station
East Bush
Roslin
Edinburgh EH25 9RE

Professor Neil Douglas
Dept of Medicine
Respiratory Medicine Unit
Royal Infirmary
Edinburgh EH3 9YW

Dr Michael Duchen
Dept of Physiology
University College London
Gower Street
London WC1E 6BT

Ms Isobel Dundas
Portex Anaesthesia Intensive Therapy
and Respiratory Medicine Unit
Institute of Child Health
Level 6, Cardiac Wing
30 Guilford Street
London WC1N 1EH

Mr Steven Duneclift
Dept of Physiology
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Professor Christopher Edwards
Governor
The Wellcome Trust
183 Euston Road
London NW1 2BE

Professor Martin Evans
Wellcome/CRC Institute
Tennis Court Road
Cambridge CB2 1QR

Dr Shelley Folkard
Lung Research Group
Southmead Hospital
Medical School Unit
Westbury On Trym
Bristol BS10 5NB

Mr Gregor Fyfe
Dept of Child Health
Ninewells Hospital and Medical School
Dundee DD1 9SY

Dr William Gardner
Dept of Thoracic Medicine
King's College School of Medicine
Bessemer Road
London SE5 9PJ

Mrs Mhairi Gilmour
Dept of Obstetrics and Gynaecology
University of Dundee Medical School
Ninewells Hospital
Dundee DD1 9SY

Dr Catherine Goddard
Wellcome/CRC Institute
Tennis Court Road
Cambridge CB2 1QR

Professor Abraham Guz
Charing Cross and Westminster Hospital
Room 19; 11N
Fulham Palace Road
London W6 8RF

Mr Dominic Hague
Dept of Allergy and Respiratory Medicine
UMDS of St Thomas' Hospital
4th Floor, North Wing
Lambeth Palace Road
London SE1 7EH

Dr Ruth Hamilton
Nuffield Dept of Anaesthetics
Radcliffe Infirmary
Woodstock Road
Oxford OX2 6HE

Mrs Anne Hancock
Lung Research Group
Medical School Unit
Southmead Hospital
Westbury On Trym
Bristol BS10 5NB

Professor Mark Hanson
Dept of Obstetrics and Gynaecology
University College London Medical School
86–96 Chenies Mews
London WC1E 6HX

Dr Gordon Harkiss
Dept of Veterinary Pathology
Royal (Dick) School of Veterinary Studies
University of Edinburgh
Summerhall
Edinburgh EH9 1QH

Dr Helen Harty
Dept of Medicine
Charing Cross and Westminster Medical School
Fulham Palace Road
London W6 8RF

Professor Brian Harvey
Dept of Physiology
Wellcome Cellular Physiology Research Unit
University College Cork
Republic of Ireland

Mr Christopher Hatton
Institute for Cardiovascular Research
University of Leeds
Leeds LS2 9JJ

Dr Paul Hellewell
Dept of Applied Pharmacology
National Heart and Lung Institute
Imperial College
Dovehouse Street
London SW3 6LY

Miss Georgie Hewitt
Conference Secretary
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Stuart Hirst
Dept of Allergy and Respiratory Medicine
UMDS of St Thomas' Hospital
4th Floor, North Wing
Lambeth Palace Road
London SE1 7EH

Dr Gerard Hoyne
Dept of Biology
Infection and Immunity Section
Imperial College of Science, Technology
and Medicine
Prince Consort Road
London SW7 2BB

Dr Robert Hume
Dept of Child Health
University of Dundee Medical School
Ninewells Hospital
Dundee DD1 9SY

Dr Tracy Hussell
Dept of Respiratory Medicine
St Mary's Hospital Medical School
Imperial College
Norfolk Place
London W2 1PG

Dr Clare Huxley
Dept of Biochemistry and Molecular Genetics
St Mary's Hospital Medical School
Norfolk Place
London W2 1PG

Dr Alan Jackson
National Heart and Lung Institute
Manresa Road
London SW3 6LR

Dr Peter Jose
Dept of Applied Pharmacology
National Heart and Lung Institute
Dovehouse Street
London SW3 6LY

Professor Noor Kalsheker
Dept of Clinical Laboratory Sciences
Division of Clinical Chemistry
Queens Medical Centre
University Hospital
Nottingham NG7 2UH

Dr Paul Kemp
Dept of Child Health
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY

Dr Janice Kitson
Scientific Officer
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Ger Eng Kwan-Lim
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Prem Kumar
Dept of Physiology
Medical School
University of Birmingham
Birmingham B15 2TT

Professor Jonathan Lamb
Dept of Biology
Infection and Immunity Section
Imperial College of Science, Technology
and Medicine
Prince Consort Road
London SW7 2BB

Miss Rachel Landauer
Dept of Physiology
University of Birmingham
Medical School
Birmingham B15 2TT

Dr Donald Lane
Osler Chest Unit
Churchill Hospital
Headington
Oxford OX3 7LJ

Dr Sang-Jin Lee
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Angus Leeming
Centre for Biological and Medical Systems
Imperial College for Science, Technology
and Medicine
London SW7 2BX

Dr Michael Lethem
Dept of Pharmacy
University of Brighton
Lewes Road
Moulsecoomb
Brighton BN2 4GJ

Ms Ania Manson
Dept of Biochemistry and Molecular Genetics
St Mary's Hospital Medical School
Norfolk Place
London W2 1PG

Dr Michael Markiewicz
Academic Child Health
Chelsea and Westminster Hospital
369 Fulham Road
London SW10 9NH

Ms Lindsay Marshall
Dept of Child Health
Ninewells Hospital and Medical School
Dundee DD1 9SY

Mr Peter Marsters
Dept of Clinical Laboratory Sciences
Division of Clinical Chemistry
Queens Medical Centre
University Hospital
Nottingham NG7 2UH

Miss Mary Martin
National Heart and Lung Institute
Manresa Road
London SW3 6LR

Miss Kerri McIntosh
Dept of Obstetrics and Gynaecology
University of Dundee Medical School
Ninewells Hospital
Dundee DD1 9SY

Dr Anil Mehta
Dept of Child Health
Ninewells Hospital and Medical School
Dundee DD1 9SY

Dr Ann Millar
Lung Research Group
Medical School Unit
Southmead Hospital
Westbury On Trym
Bristol BS10 5NB

Professor Hugh Miller
Dept of Veterinary Clinical Studies
Royal (Dick) School of Veterinary Studies
Veterinary Field Station
East Bush
Roslin
Edinburgh EH25 9RE

Dr Rohit Mistry
Dept of Molecular Genetics
Institute of Biomedical and Life Sciences
University of Glasgow
Pontecorvo Building
Anderson College
56 Dumbarton Rd
Glasgow G11 6N

Dr Miriam Moffatt
Nuffield Dept of Clinical Medicine
John Radcliffe Hospital
Headington
Oxford OX3 9DU

Dr Mart Mojet
Dept of Physiology
University College London
Gower St
London WC1E 6BT

Ms Denise Moore
Dept of Experimental Medicine
University of Sheffield Medical School
Beech Hill Road
Sheffield S10 2RX

Dr Kevin Morgan
Dept of Clinical Laboratory Sciences
Division of Clinical Chemistry
Queens Medical Centre
University Hospital
Nottingham NG7 2UH

Dr Mary Morrell
Dept of Preventive Medicine
University of Wisconsin
504 N Walnut
Madison WI 53705–2368
USA

Dr Ian Mortimore
Dept of Medicine
Respiratory Medicine Unit
Royal Infirmary
Edinburgh EH3 9YW

Dr Richmond Muino
Dept of Child Health
Ninewells Hospital and Medical School
Dundee DD1 9SY

Dr Somnath Mukhopadhyay
Dept of Child Health
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY

Dr Kevin Murphy
Dept of Medicine
Charing Cross and Westminster Hospital
Fulham Palace Road
London W6 8RF

Dr Ray Noble
Dept of Obstetrics and Gynaecology
University College London Medical School
86–96 Chenies Mews
London WC1E 6HX

Dr Piers Nye
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Roddy O'Donnell
Dept of Respiratory Medicine
St Mary's Hospital Medical School
Imperial College
Norfolk Place
London W2 1PG

Professor Richard Olver
Dept of Child Health
Ninewells Hospital and Medical School
Dundee DD1 9SY

Dr Peter Openshaw
Dept of Respiratory Medicine
St Mary's Hospital Medical School
Imperial College
Norfolk Place
London W2 1PG

Professor Sir Stanley Peart
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Chris Peers
Institute for Cardiovascular Research
University of Leeds
Leeds LS2 9JJ

Dr David Pepper
Dept of Physiology
Medical School
University of Birmingham
Birmingham B15 2TT

Dr Mary Phillips
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Marc Poulin
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Sara Rankin
Dept of Applied Pharmacology
National Heart and Lung Institute
Dovehouse Street
London SW3 6LY

Dr Pamela Reid
Scientific Officer
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Peter Robbins
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Tom Robertson
Dept of Allergy and Respiratory Medicine
UMDS of St Thomas' Hospital
4th Floor, North Wing
Lambeth Palace Road
London SE1 7EH

Dr William Roche
Dept of Pathology
University of Southampton
South Block, Level E
Southampton General Hospital
Southampton SO16 6YD

Dr Jonathan Round
Dept of Child Health
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Dr Sigmar Saisch
Maudsley Hospital
Denmark Hill
London SE5

Dr George Santis
Dept of Allergy and Respiratory Medicine
UMDS of Guy's Hospital
4th Floor, Hunts House
London SE1 9RT

Professor Robert Schroter
Centre for Biological and Medical Systems
Imperial College for Science, Technology
and Medicine
London SW7 2BX

Dr Laurence Smaje
The Wellcome Centre for Medical Science
210 Euston Road
London NW1 2BE

Dr Vladimir Snetkov
Dept of Allergy and Respiratory Medicine
UMDS of St Thomas' Hospital
London SE1 7EH

Professor Michael Spyer
Dept of Physiology
University College London
Gower Street
London WC1E 6BT

Mr Richard Stephens
Dept of Child Health
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Dr John Stephenson
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Myra Stern
National Heart and Lung Institute
Emmanuel Kaye Building
Manresa Road
London SW3 6LR

Mrs Jilly Steward
Meetings and Travel Manager
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Jacob Sweiry
Scientific Officer
The Wellcome Trust
183 Euston Road
London NW1 2BE

Mr John Tansley
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Terry Tetley
Dept of Medicine
Charing Cross and Westminster Medical School
Fulham Palace Road
London W6 8RF

Dr Mark Upton
Dept of General Practice
Woodside Health Centre
Barr Street
Glasgow G20 7LR

Dr Valerie Urbach
Dept of Physiology
Wellcome Cellular Physiology Research Unit
University College Cork
Republic of Ireland

Mr John Varley
Dept of Human Biology
University of Leeds
School of Medicine
Leeds LS2

Dr Georges Vassaux
Dept of Biochemistry and Molecular Genetics
St Mary's Hospital Medical School
Norfolk Place
London W2 1PG

Dr Richard Vaughan-Jones
Laboratory of Physiology
University of Oxford
Parks Road
Oxford OX1 3PT

Dr Andrew Walley
Nuffield Dept of Clinical Medicine
John Radcliffe Hospital
Headington
Oxford OX3 9DU

Professor Mark Walport
Rheumatology Unit
Royal Postgraduate Medical School
Du Cane Road
London W12 0NN

Ms Deirdre Walsh
Dept of Physiology
Wellcome Cellular Physiology Research Unit
University College Cork
Republic of Ireland

Professor Dafydd Walters
Dept of Child Health
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Dr Jeremy Ward
Dept of Allergy and Respiratory Medicine
UMDS of St Thomas' Hospital
4th Floor, North Wing
Lambeth Palace Road
London SE1 7EH

Dr Malcolm Watson
School of Pharmacy and Pharmacology
University of Bath
Bath BA2 7AY

Dr Ursula Wells
Dept of Physiology
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Miss Anna Maria White
School of Pharmacy and Pharmacology
University of Bath
Bath BA2 7AY

Mr Jonathan Whiteley
Nuffield Dept of Anaesthetics
Radcliffe Infirmary
Woodstock Road
Oxford OX2 6HE

Professor John Widdicombe
Dept of Physiology
St George's Hospital Medical School
Cranmer Terrace
London SW17 0RE

Dr Michael Wilkinson
The Wellcome Trust
183 Euston Road
London NW1 2BE

Professor Timothy Williams
Dept of Applied Pharmacology
National Heart and Lung Institute
Dovehouse Street
London SW3 6LY

Dr Robert Wilson
National Heart and Lung Institute
Manresa Road
London SW3 6LR

Dr Terry Woolley
Dept of Applied Pharmacology
National Heart and Lung Institute
Imperial College
Dovehouse Street
London SW3 6LY

Dr Paul Wymer
Head of Communication and Education
The Wellcome Trust
183 Euston Road
London NW1 2BE

Dr Shaoli Zhang
Dept of Pathology
University of Southampton
South Block, Level E
Southampton General Hospital
Southampton SO16 6YD

The Respiratory System in Health and Disease

Meeting Questionnaire

Q1. Which of the following best describes your involvement with todays meeting ?

- ☐ Gave an oral presentation
- ☐ Part of a group associated with an oral presentation
- ☐ Part of a group associated with a poster presentation
- ☐ Gave a plenary lecture
- ☐ None of these

Q2. What is your level of experience within the field ?

- ☐ Post-graduate
- ☐ Department/Faculty member
- ☐ Post-doctoral
- ☐ Head of Department/Faculty
- ☐ Other

Q3. Do you work in clinical or basic science ?

☐ Clinical ☐ Basic

Q4. What is your opinion of the days activities as a whole?

☐ Very poor ☐ Poor ☐ Good ☐ Very good

Q5. Which particular aspect of the day have you found MOST useful ?

Q6. Which particular aspect of the day have you found LEAST useful ?

Q7. When coming to the meeting which of the following aspects did you anticipate it would enable you to do. Which of these aspects do you feel the meeting has enabled you to do?

	ANTICIPATED Tick ALL that apply	ENABLED Tick ALL that apply	MOST IMPORTANT Tick ONE only
Q7a. To discover what other scientists are doing in different areas of research	☐	☐	☐
Q7b. To disseminate information on your own areas of research	☐	☐	☐
Q7c. To receive feedback on issues arising from your own research.	☐	☐	☐
Q7d. To attend a forum for younger scientists to present their work	☐	☐	☐
Q7f. To develop collaborative projects with other participants	☐	☐	☐
Q7g. To learn something of importance that you may otherwise have missed	☐	☐	☐

Q8. If you feel the meeting has not ENABLED you to achieve any of the aspects above that you ANTICIPATED please explain the reasons below.

Q9. Please indicate whether you feel each of the following were allocated too much, too little or about the right amount of time.

	Too little	About right	Too much
Q9a. Plenary lectures	☐	☐	☐
Q9b. Oral presentations	☐	☐	☐
Q9c. Poster presentations	☐	☐	☐
Q9d. Total duration of the meeting	☐	☐	☐

Q10. What is your view of the overall standard of the presentations ?

	Very poor	Poor	Good	Very good
Q10a. Oral presentations	☐	☐	☐	☐
Q10b. Poster presentations	☐	☐	☐	☐
Q10c. Plenary lectures	☐	☐	☐	☐

Q11. Were the plenary lectures sucessful in giving an overall view of the subject area ?

☐ Yes ☐ No

Q12. What is your opinion of the range of research presented in todays meeting ?

Too broad ☐ About right ☐ Too Narrow ☐

Q13. What is your opinion of the following aspects of the organisation of the meeting `?

	Very poor	Poor	Good	Very good
Q13a. Catering	☐	☐	☐	☐
Q13b. Advance information for the meeting	☐	☐	☐	☐

Q14. Finally, is there anything else that you wish to add about any aspect of today's proceedings ?

Thank-you for your co-operation. All responses will be treated in confidence and we hope will be useful to us in organising future events.

Please hand this questionnaire in as you leave or send it to:
Ian Muchamore, Scientific Communication Officer, The Wellcome Centre for Medical Science, 210 Euston Road, London. NW1 2BE