SHORT PAPER

Preliminary findings of quorum signal molecules in clinically stable lung allograft recipients

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Background: Infection with bacteria such as *Pseudomonas* is common in lung allograft recipients, particularly during chronic rejection. Analysis of sputum samples from patients with cystic fibrosis infected with *Pseudomonas aeruginosa* or *Burkholderia cepacia* has indicated the presence of bacterial *N*-acylhomoserine lactones (AHLs) quorum sensing signalling molecules. AHLs not only control the expression of bacterial virulence genes but are also involved in stimulating the maturation of antibiotic resistant biofilms and host chemokine release. It was hypothesised that AHLs may be detected even in clinically stable lung transplant recipients free of clinical infection or rejection.

Methods: Three 60 ml samples of bronchoalveolar lavage (BAL) fluid were taken from nine stable lung transplant recipients 3–12 months after transplantation. Detection of AHLs was carried out on dichloromethane extracted supernatants using the bioluminescence based AHL reporter plasmid pSB1075. This responds to the presence of AHLs with long acyl chains (C10–C14), generating light. Synthetic AHLs were included as positive controls.

Results: Five of the nine BAL fluid supernatants exhibited AHL activity, suggesting the presence of AHLs with long N-acyl chains. There was no correlation between the levels of AHLs detected or their absence and BAL fluid microbiology or diagnosis before transplantation.

Conclusions: This is the first evidence for the presence of AHL quorum sensing signals in human lung allograft recipients, even in subjects with no rejection or apparent infection. Further longitudinal follow up of these preliminary findings is required to elucidate potential links with infection, rejection, and allograft deterioration.

Lung transplantation is an accepted treatment for advanced lung disease and is particularly important for long term survival in young people with progressive disease.[1] Chronic lung allograft rejection is destructive, scars the airways, and leads to progressive irreversible loss of lung function—the bronchiolitis obliterans syndrome (BOS). BOS is the most common cause of morbidity and mortality in lung transplant recipients after the first few months, with a highly negative impact upon all programmes. The aetiology of BOS remains poorly understood, but candidate risk factors include infection, acute rejection, and inflammation.[1]

In recent years it has been established that many different bacteria coordinate gene expression in a cell density dependent manner using small diffusible signalling molecules, a phenomenon termed "quorum sensing".[2] In Gram negative bacteria the most intensively studied quorum sensing systems use *N*-acylhomoserine lactones (AHLs) as intercellular signalling molecules. Of particular relevance, the two major pathogens in cystic fibrosis (CF)—*Pseudomonas aeruginosa* and *Burkholderia cepacia*—communicate in this way. *P aeruginosa* uses mainly three AHLs—namely, *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), *N*-butanoyl-L-homoserine lactone (C4-HSL), and *N*-hexanoyl-L-homoserine lactone (C6-HSL).[2] Importantly, cell-cell signalling in *P aeruginosa* is involved in the regulation of a number of virulence determinants such as elastase which contributes to the deterioration of lung function in colonised CF patients.[2]

Very recent studies in patients with CF have identified the presence of quorum signal molecules in sputum samples of patients colonised by *P aeruginosa* and *B cepacia*.[3] Furthermore, in a description of two patients these signalling molecules were detected in central and peripheral lung tissue from patients colonised by *P aeruginosa*.[4] Studies using *P aeruginosa* isolated from patients with CF showed that the formation of biofilms in the lung by this bacterium correlates with the production of AHLs ex vivo.[5]

Pseudomonas is frequently isolated from lung allograft recipients and especially those with BOS. Although it has been suggested that infections caused by bacteria such as *P aeruginosa* may be linked to BOS, there is no evidence supporting the involvement of AHLs in this process. This paper describes a preliminary study which shows that AHL quorum sensing signals can be detected in human lung allografts from patients without BOS, with and without conventional clinical evidence of infection.

METHODS

Three 60 ml samples of bronchoalveolar lavage (BAL) fluid were taken from nine stable non-smoking lung transplant recipients (table 1) as previously described.[6] The selection and procurement of the pulmonary donor,[7] immunosuppression,[7] bronchoscopy and BAL, and preparation of cell free BAL supernatants[6] were carried out as in previous studies.

Detection of *N*-acylhomoserine lactones (AHLs) in BAL fluid

BAL fluid supernatants (900 µl) were extracted twice with equal volumes of HPLC grade dichloromethane and evaporated to dryness overnight as described by Middleton *et al*.[3] The extracts were reconstituted in 200 µl acetonitrile and 5 µl was analysed by thin layer chromatography. To detect the presence of long chain (C10–14) AHLs, a bioluminescence based *E coli* biosensor strain (*E coli* JM109) harbouring the AHL reporter plasmid pSB1075 was used.[3] In all cases light emission resulting from the detection of AHLs was captured using a Berthold LB980 photon video camera (E G and G Berthold UK Ltd, Milton Keynes, UK). Synthetic AHLs were

Table 1 Clinical details of the study population at the time of bronchoscopy and results of the quorum sensing molecule assays

SN No	Pre allograft diagnosis/operation type	Microbiology pretransplantation from expectorate	BAL microbiology contemporaneous with AHL assay	Months after treatment	Immunosuppression	Evidence of rejection	BOS score	BAL supernatant *v* pSB1075
1	LAM/single	–ve	–ve	3	Triple	Nil	0	+
2	Emphysema/single	–ve	–ve (previous *Aspergillus*)	3	Triple	Nil	0	++
3	α-1AT/single	*P aeruginosa*; *Alkaligenes xyolosoxidans*	–ve	12	Triple	Nil	0	–
4	CF/sequential single	*P aeruginosa*; *Pseudomonas* spp	*P putida*; *Comamonos testosteroni*	3	Triple (+ antibiotic)	Nil	0	+
5	Bronchiectasis/sequential single	*P aeruginosa*	Mucopurulent *Candida albicans*	3	Triple (+ antibiotic)	Nil	0	++
6	PPH/heart-lung	–ve	–ve	3	Triple	Nil	0	+++*
8	CF/sequential single	No sputum	*P aeruginosa*	3	Triple (+ antibiotic)	Nil	0	–
9	CF/sequential single	*P aeruginosa*	–ve	3	Triple (+ antibiotic)	Nil	0	–
11	CF/sequential single	–ve	*Pseudomonas* spp	3	Triple (+ antibiotic)	Nil	0	–

SN No=supernatant number; a supernatant randomisation number generated at the AHL assays (University of Nottingham) so that the assays were performed blinded to patient details; microbiology pretransplantation from expectorate=recipient microbiology measured from expectorated sputum before transplantation; microbiology contemporaneous with AHL assay=standard BAL microbiology performed on the same BAL fluid samples used in the AHL assays; LAM=lymphangiomatosis; PPH=primary pulmonary hypertension; CF=cystic fibrosis; α-1AT=α_1-antitrypsin deficiency.
Immunosuppression: triple=standard triple immunosuppression with cyclosporin, prednisone and azathioprine; + antibiotic indicates use of intravenous induction with prophylactic antibiotic therapy supplemented with subsequent nebulised therapy. Used in all subjects transplanted because of septic lung disease such as end stage CF.[7]
pSB1075 is a bioluminescence reporter that responds to AHLs with long acyl chains (C10–C14) by generating light.
*Semi-quantitative level of AHLs detected (+, ++, +++).

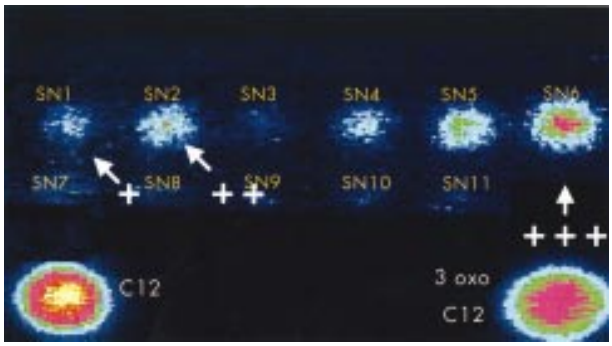

Figure 1 Representative bioluminescence capture resulting from the activation of the *N*-acylhomoserine lactone (AHL) biosensor pSB1075. SN1, 2 and 6=positive patient samples denoted by light emission (white arrows); C12=*N*-dodecanoyl-L-homoserine lactone standard (10 μg/ml); 3 oxo C12=*N*-(3-oxododecanoyl)-L-homoserine lactone standard (1 μg/ml).

used as positive controls. The investigator performing the AHL assay was blind to the patient clinical data.

RESULTS

The clinical details of the study population at the time of the bronchoscopy are shown in table 1, together with the results of clinical microbiological assessment.

BAL was successfully performed in all the lung transplant recipients (median return 90 ml, range 66–120 ml). Neutrophil counts were variable and moderately raised, confirming our previous results (median 3%, range 0–82%).[6 8 9]

From the nine extracted BAL fluid supernatants analysed, five exhibited AHL activity with the *E coli* pSB1075 biosensor, suggesting the presence of long chain AHLs (fig 1, table 1). Interestingly, there was no correlation between the level of AHLs detected (+, ++, +++) or their absence and pretransplant diagnosis, positive BAL microbiological culture (table 1), or lung function.

DISCUSSION

In this study we provide the first evidence of quorum signals in a series of clinically stable human lung allograft recipients, with AHL activity detectable even in subjects with no evidence of rejection, BOS, or infection (clinically or following BAL microbiology).

Many bacteria coordinate gene expression and hence phenotype in a cell density dependent manner employing quorum sensing signal molecules. Of these, the AHLs measured in this study have been intensely investigated in *P aeruginosa* and *B cepacia* infection of patients with CF.[3 5]

This study supports previous findings that pathophysiological changes are present even in clinically stable lung allograft recipients.[6 8 9] Our reports of increased airway neutrophil and interleukin (IL)-8 levels in clinically stable allograft recipients are potentially complementary to the present study, and we have also shown increased levels of soluble CD14 in BAL fluid.[6 8 9] CD14 is a surface protein, shed following activation, which is found on macrophages and activated neutrophils. It serves as the cellular receptor for lipopolysaccharide, a major component of Gram negative bacteria such as *P aeruginosa* and *B cepacia* which produce AHLs.

AHL activity may be particularly strategic, exhibiting pharmacological properties and modulating T cell and macrophage function, as well as being involved in control of virulence factors and biofilm formation and eliciting host IL-8 production.[2 10] This is the first description of quorum signals in lung transplantation, and our findings are limited by the preliminary cross sectional nature of this study. Longitudinal studies are therefore required to track AHL levels and to elucidate potential relationships with infection, BOS, and the pathophysiological deterioration of lung allografts.

The inclusion of rapid DNA based identification methods for *P aeruginosa* and other bacteria in allografts may be useful in future studies. There is also a need to include appropriate patient controls. This type of study based on the detection of AHL signal molecules may lead to the establishment of a link between the presence of AHLs in allografts and BOS. This may aid the search for novel treatments which interfere with the production and mode of action of these quorum sensing signal molecules.

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Conflict of interest: none.

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LUNG ALERT

Antibiotics for RTIs in adult primary care

▲ Steinman MA, Landefeld CS, Gonzales R. Predictors of broad-spectrum antibiotic prescribing for acute respiratory tract infections in adult primary care. *JAMA* 2003;**289**:719–25

This paper makes a useful contribution to the debate surrounding the prescription of antibiotics for non-pneumonic acute respiratory tract infections (RTIs). Using a US community based outpatient physician database, the authors assessed frequency of prescription of antibiotics, use of broad spectrum antibiotics, and factors predicting their use. They found that, despite little evidence of therapeutic benefit, 63% of patients with an acute RTI were prescribed antibacterial agents and 54% of the antibiotics given were broad spectrum. The rate of prescription varied significantly between geographical regions and with type of physician, race, and ownership of insurance.

Although the methodology of the study means that factors arguably important in determining whether antibacterial drugs should be prescribed cannot be fully determined—in particular, the presence of significant co-morbidity and severity of illness—the data are still compelling. Approximately half of the subjects presenting with a common cold or non-specific upper respiratory tract infection were given antibiotics and these were broad spectrum in half of the cases. The authors discuss the multiple, often interacting, factors which determine whether an antibiotic is prescribed at the time of a consultation and further comment on ways to impact on the prescription rate. This paper is timely in light of the increasing rates of antimicrobial resistance and complications related to broad spectrum antibiotic prescription.

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