Incidence of common pyocin types of *Pseudomonas aeruginosa* from patients with cystic fibrosis and chronic airways diseases

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Summary. We sought evidence to determine if particular strains of *Pseudomonas aeruginosa* have a predilection for pulmonary colonisation in patients with cystic fibrosis (CF). The incidence of common pyocin types in non-CF isolates (74%) was similar to that noted in previous reports but differed significantly ($\chi^2 = 16.7$, $p < 0.001$) from the incidence of 40% observed in CF isolates. A retrospective analysis of respiratory isolates also indicated a relatively low incidence of common pyocin types (44%) in isolates from non-CF patients with chronic airways diseases and this incidence also differed significantly from that observed (73%) in other respiratory isolates from patients in the same hospital. These observations suggest that a subpopulation of *P. aeruginosa* exists which has a predilection for pulmonary colonisation in CF and other chronic pulmonary diseases and may assist in identification of factors affecting bacterial colonisation.

Introduction

Epidemiological studies of the association of *Pseudomonas aeruginosa* and chronic pulmonary colonisation in patients with cystic fibrosis (CF) are greatly helped by typing techniques which indicate the clonal relationship of individual strains. Serotyping, based on O-antigen specificity is not useful for characterisation of *P. aeruginosa* from patients with CF because of alterations in bacterial lipopolysaccharide which are characteristically observed in chronic pulmonary colonisation.¹ Pyocin typing^{2,3} which relies on the production and detection of chromosomally determined bacteriocins, DNA probe analysis by Southern blot hybridisation with DNA fragments from the exotoxin A gene,⁴ and genome fingerprinting by field inversion gel electrophoresis⁵ have been used successfully to determine two important epidemiological factors in patients with CF.⁶ Firstly, the majority of patients remain colonised with a single strain of *P. aeruginosa* which gradually evolves to exhibit a range of phenotypic properties including mucoidy due to derepressed alginate biosynthesis, loss of O-antigen specificity, serum sensitivity and hypersusceptibility to a range of antibiotics. Secondly, cross-infection is rare, except between siblings with CF.

The purpose of the present study was to address a third epidemiological consideration—to determine if a subpopulation of particular strains of *P. aeruginosa* have a predilection for chronic colonisation of CF patients. The rationale for the study had emerged from two sources. First, from our ongoing longitudinal studies with pyocin typing which indicated that cross-infection with the same strain was rare, and that *P. aeruginosa* isolated from patients with CF appeared to have a different pyocin type distribution compared to non-CF isolates. Second, from the evidence from closely documented episodes of primary colonisation by non-mucoid *P. aeruginosa* in patients with CF which suggested that in environments containing multiple strains of *P. aeruginosa* some strains appeared to have enhanced ability to colonise patients.⁷

Materials and methods

Bacteria

A collection of 304 non-CF isolates of *P. aeruginosa* comprised all isolates from sputum and other specimens and anatomical sites in patients treated in three Edinburgh hospitals during 1987. The series of 69 CF isolates of *P. aeruginosa* was from the sputum of patients attending the Edinburgh CF clinics. Subsequently, a retrospective analysis of pyocin type distribution was performed on all respiratory isolates of *P. aeruginosa* which had been isolated from non-CF patients in one hospital during the

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period 1986–1988; these isolates comprised 60 strains from patients with chronic airways diseases (CAD) and 52 from patients colonised with *P. aeruginosa* post-operatively. Isolates were identified as *P. aeruginosa* by colonial morphology and production of characteristic pigmentation of Pseudomonas Isolation Agar (Difco, 0927–01); non-pigmented isolates were identified by a positive oxidase reaction⁸ and with API 20NE kits (API Laboratory Products Ltd, Basingstoke).

Pyocin typing

Pyocin typing was performed by our revised technique³ which incorporates the original type patterns designated earlier in this laboratory. Repeat specimens from the same patient were excluded, as were multiple isolations of a single epidemic strain. A χ^2 statistical analysis was used to determine significant differences in the pyocin type distribution of the different populations; $p < 0.05$ was considered to represent a statistically significant difference.

Results

Analysis of the distribution of pyocin types revealed a lower incidence of common pyocin types in patients with CF than in isolates from other patients (table I). Amongst 69 isolates from patients with CF, only 28 (40%) produced the common type patterns 1, 3, 5 or 10 based on inhibition of the original set of eight indicator strains. No single pyocin type accounted for a significant proportion of the remaining 41 strains which were distributed amongst 25 pyocin types.

When the incidence of the common type patterns 1, 3, 5 and 10 in CF isolates was compared with that of the non-CF isolates from the three individual hospitals, the results for hospitals 1 and 2 (74% and 70% respectively) were similar to previous reports and differed significantly from the CF isolates

($\chi^2 = 16.69$, $p < 0.001$ and $\chi^2 = 13.03$, $p < 0.001$ respectively) whereas isolates from hospital 3 with an incidence of types 1, 3, 5 and 10 at 58% fell midway between the other groups; however, this incidence was still significantly different from that amongst the CF isolates ($\chi^2 = 5.95$; $p < 0.02$; table I). Although the isolates from patients attending hospital 3 did not contain any strains from patients with CF, further analysis of the sources of *P. aeruginosa* in hospital 3 showed a large proportion of isolates from sputum samples from patients with pulmonary infections. Sputum isolations of *P. aeruginosa* accounted for 39% of all isolates from hospital 3 compared with 7% of isolates from hospitals 1 and 2. Subsequently, in a retrospective study, when all sputum isolates from hospital 3 were excluded from analysis, the incidence of common pyocin types in non-sputum isolates in hospital 3 was 73%; thus, there was no significant difference in the incidence of common pyocin types in all isolates of *P. aeruginosa* in hospital 1 and 2 and non-sputum isolates in hospital 3 ($\chi^2 = 1.38$, $0.5 > p > 0.1$ and $\chi^2 = 0.23$, $p > 0.5$ respectively). This observation suggested that the relatively low incidence of common types 1, 3, 5 and 10 observed in the CF isolates might also be associated with pulmonary colonisation in non-CF patients. This hypothesis was confirmed when a comparison was made of the incidence of common types in patients with CF and sputum isolates obtained from hospital 3 from post-operative patients and patients with chronic airways disease colonised by *P. aeruginosa*. This analysis (table II) indicated no significant difference in the incidence of common pyocin types of *P. aeruginosa* in CF isolates and in isolates from non-CF patients with chronic airways diseases including chronic bronchitis, bronchiectasis, asthma and interstitial lung disease; however, the

Table I. Incidence of pyocin types 1, 3, 5 and 10 of *P. aeruginosa* in isolates from sputum from patients with CF, compared with all isolates from non-CF patients in three Edinburgh hospitals

Source	Incidence (%) of pyocin types 1, 3, 5 and 10	p value*
Patients with CF	28 (40)	—
Patients without CF		
in hospital 1	55 (74)	< 0.001
in hospital 2	56 (70)	< 0.001
in hospital 3	88 (58)	< 0.02

* Compared with CF isolates.

Table II. Incidence of pyocin types 1, 3, 5 and 10 of *P. aeruginosa* in isolates from sputum from patients with CF compared with sputum isolates from patients with chronic airways disease (CAD) or post-operative respiratory infection

Source	Incidence (%) of pyocin types 1, 3, 5 and 10	p value*
Patients with CF	28 (40)	—
Patients with CAD	26 (44)	< 0.50
Patients with post-operative infection	38 (73)	< 0.001

* Compared with CF isolates.

incidence of common pyocin types in acute respiratory infections of post-operative patients was similar to that observed in hospitals 1 and 2.

Detection of the production of low molecular weight S-type pyocins greatly increases the discrimination of pyocin typing.^{2,3} Comparison of the production of S-type pyocin activity in the CF isolates and those isolates from non-CF patients treated in the three hospitals indicated that there was no significant difference in the proportion of strains producing S-type activity nor in the S-type inhibition patterns observed with the indicator strains.

Discussion

The evidence from this study indicates that strains of *P. aeruginosa* responsible for pulmonary colonisation in patients with CF and other chronic airways diseases exhibit an incidence of common pyocin types which is significantly lower than noted in previously published reports from world-wide studies in which the same pyocin typing system was used. In these reports^{2,9-12} the predominance of pyocin types 1, 3, 5 and 10 is clear and ranges from 58% to 89% not only amongst clinical isolates, but also amongst non-clinical isolates from food and other environmental sources.¹⁰ Indeed, the fact that early studies showed that pyocin types 1, 3, 5 and 10 accounted for the majority of *P. aeruginosa* isolates on a world-wide basis was the rationale behind our introduction of an additional set of indicator strains¹³ and the suggestion that detection of S-type pyocin activity allowed further subdivision of these common types and hence improved the discriminative potential of pyocin typing to characterise individual isolates of *P. aeruginosa*.^{2,3}

The relatively low incidence of common pyocin types in *P. aeruginosa* isolates from infections in patients with CF and other chronic airways diseases suggests that a subpopulation of *P. aeruginosa*, with as yet unidentified colonising factors, might be responsible for pulmonary colonisation in these patients.

Our results cannot be explained as a peculiarity of the Edinburgh CF clinics since the low incidence of types 1, 3, 5 and 10 was also observed in hospital 3 in isolates from non-CF patients with chronic airways diseases. In addition, a preliminary report from a current independent study on the use of pyocin typing to characterise CF isolates of *P. aeruginosa* has confirmed the relatively low incidence of the common pyocin types in another geographical area (T. L. Pitt, personal communication).

The reasons underlying the predominance of relatively uncommon pyocin types of *P. aeruginosa* in chronic pulmonary colonisation could be associated with factors involved in pyocin production or with the properties of pyocins *per se*. Bacteriocin production and susceptibility are arguably more commonly observed in strains of *P. aeruginosa* than in most other bacterial species; furthermore, the classes of bacteriocins produced represent the combined range found in other species. Phage-tail-like R and F pyocins¹⁴⁻¹⁶ are produced by more than 90% of clinical isolates and colicin-like, trypsin-sensitive S pyocins^{2,17} are produced by over 70% of strains,³ susceptibility to these bacteriocins occurs in 100% of strains. Some strains of *P. aeruginosa* also produce a class of low molecular weight, trypsin-resistant pyocins which resemble the microcins of the enterobacteria.¹⁸ Little is known of the role of pyocins *in vivo* or of their cell surface receptors.¹⁹ From this study, however, we can reasonably conclude that the S pyocins, which share receptors with *Pseudomonas* siderophores,^{19,20} and the microcin-like pyocins, are not involved in pulmonary colonisation.

Alternatively, the apparent predilection of uncommon pyocin types of *P. aeruginosa* to establish chronic pulmonary colonisation might merely indicate a subpopulation of strains with properties, which enhance their ability to establish chronic colonisation, but which are independent of pyocin activity. Since all pyocins (R, F, S and microcin-like) studied to date are encoded by chromosomal genes^{21,22} (findings further confirmed by our own unpublished observations) we could speculate that genes regulating colonising factors might be closely linked or co-regulated with determinants encoding uncommon pyocin type patterns. However, the range of pyocins produced singly or in combinations by strains of *P. aeruginosa*, is considerable and the role of individual pyocins in relation to the type patterns of inhibition against the indicator strains is little understood. It may be significant that the gene responsible for pyocin R2, which is the major pyocin responsible for production of the common type patterns 1 and 10, is located at 23 min on the PAO chromosome, near loci regulating several *P. aeruginosa* virulence determinants including exotoxin A and the siderophore pyoverdinin.²³ At present, however, there is no evidence which links pyocin genes with genetic determinants for *Pseudomonas* colonising factors.

In conclusion, these studies have demonstrated a subpopulation of *P. aeruginosa* with enhanced potential to establish chronic pulmonary colonisation in patients with CF and other chronic

obstructive airways diseases. The basis for this subpopulation is unclear but may include pyocin activity, the genetic determinants for pyocins or other colonising factors. It will be interesting to observe if a similar subpopulation is identified from

epidemiological studies with DNA-based typing techniques.

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