Invasion by *Neisseria meningitidis* varies widely between clones and among nasopharyngeal mucosae derived from adult human hosts

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Colonization of the human nasopharynx is a feature of some species of *Neisseria*, and is a prerequisite of invasive meningococcal disease. The likelihood of colonization by *Neisseria meningitidis* varies widely between humans, and very few develop invasive disease. Explants of nasal mucosa derived from adult patients with non-allergic nasal obstruction were infected experimentally with *Neisseria* spp. At intervals over 18 h incubation, washed explants were homogenized, and viable bacteria were counted. To estimate bacterial invasion of mucosa, explants were exposed to 0.25 % sodium taurocholate for 30 s prior to homogenization. *N. meningitidis* was recovered from the mucosa and the organism invaded and replicated within the tissue, in contrast to *N. lactamica* and *N. animalis* ($n=9$, $P<0.008$). *N. meningitidis* isolates of clones ET-5, ET-37 and lineage III were recovered from and invaded tissue, but strains of clones A4, A:subgroup I, A:subgroup III and A:subgroup IV-1 did not invade ($n=6$). To measure host variation, survival of *N. meningitidis* within nasal mucosa of 40 different human donors was measured. Intra-class correlation of replicates was 0.97, but the coefficient of variation of recovered viable counts was 1335 % after 4 h and 77 % after 18 h incubation. It is concluded that the distinctive colonization and disease potential of *Neisseria* spp. may be partly a consequence of their ability to invade and survive within human nasopharyngeal mucosa, but that this is influenced greatly by genetic or environmental factors operating on the host mucosa. This is consistent with the unpredictable epidemiology of meningococcal disease.

Keywords: pathogenesis, colonization, *Neisseria lactamica*, *Neisseria animalis*

INTRODUCTION

Colonization and invasion of nasopharyngeal mucosa is the first step in the pathogenesis of meningococcal disease. Supporting evidence for this is the correlation between the prevalence of community carriage and the occurrence of meningococcal disease (Broome, 1986). During non-epidemic periods, the baseline prevalence of nasopharyngeal carriage of meningococci is 5–10 % but it is considerably higher in certain populations such as military personnel (Riordan *et al.*, 1998), and in house-

holds of cases (Greenfield & Feldman, 1967) and smokers (Stuart *et al.*, 1989). There is good evidence that host factors determine partly the success of *Neisseria meningitidis* within the nasopharynx, as some individuals appear resistant to acquisition of carriage, whilst others carry the organism chronically or intermittently (Rake, 1934). The precise site within the nasopharynx that *N. meningitidis* colonizes and invades is not known, but during natural carriage, the organism can be isolated both from the nose and from the throat (Olcen *et al.*, 1979), and can be observed by immunofluorescence deep within tonsillar tissue (Sim *et al.*, 2000).

Humans can be colonized by commensal *Neisseria*

Abbreviation: MBC, mean bactericidal concentration.

including *N. lactamica*, which rarely causes disease. Colonization by *N. lactamica* is frequent in infants but declines to relatively low rates in teenagers and adults (Cartwright *et al.*, 1987; Gold *et al.*, 1978). It is possible that *N. lactamica* displaces *N. meningitidis* from the nasopharynx in childhood and produces natural immunity against invasive meningococcal disease (Coen *et al.*, 2000) as a consequence of immunogenic epitopes shared between *N. lactamica* and *N. meningitidis*.

Organ culture permits the study of the interaction of *N. meningitidis* with a tissue which has physiologically relevant cellular and matriceal components. A number of groups have used organ culture to study biology of the interaction of *N. meningitidis* with human airway mucosa. Meningococci attach selectively to non-ciliated columnar cells, and during this process microvilli of non-ciliated cells elongate and surround the organisms. Meningococci appear to undergo parasite-directed endocytosis and are observed in subepithelial tissues adjacent to lymphoid tissue after prolonged incubation (Stephens *et al.*, 1983), though this is observed in a minority of explants (Read *et al.*, 1995). Pili and capsular polysaccharide both influence association of *N. meningitidis* with nasopharyngeal mucosa (Rayner *et al.*, 1995; Stephens *et al.*, 1993), but concurrent switching of multiple phase-variable bacterial surface components such as these, and outer-membrane proteins including Opa, appears to occur during successful invasion of this tissue (de Vries *et al.*, 1996).

In this work, a model of survival of *Neisseria* spp. within human nasopharyngeal mucosa was developed, and the success of various *Neisseria* spp. within this tissue was compared. The variation of survival of *N. meningitidis* with the mucosae of a large number of donor human tissues was then measured.

METHODS

Bacteria. Strain K454 (B15P1.7,16,L3,7,9, sulphonamide resistant) is an isolate of *N. meningitidis* taken from a child during an epidemiological investigation into an outbreak of meningococcal disease in a Gloucestershire Health District of the UK (Cartwright *et al.*, 1987). Reference isolates of *N. lactamica* and *N. animalis* were used. Also seven representative isolates derived from different clusters or clones of *N. meningitidis* were used, and these are listed in Table 1. Strains were stored in liquid nitrogen. On the experimental day, 15 µl of overnight broth culture was transferred to fresh Mueller–Hinton Broth in 6 ml screw-capped bottles and incubated to mid-exponential phase for 4 h at 37 °C on a rolling stage. The growth was centrifuged at 2000 g, washed and centrifuged three times with 1 ml phosphate-buffered saline (PBS). Twenty microlitres of the final wash diluted in PBS was examined using spectrophotometry to permit adjustment to the required inoculum.

Western blots. Expression of Opa and PilC was detected by Western blotting with (i) 4B12/C11 anti-Opa (gift of X. Nassif and P. Morand), (ii) 18P4 anti-PilC (gift of J. Moir), a rabbit polyclonal which recognizes both PilC1 and PilC2. Secondary detection was conducted using horseradish-peroxidase-conjugated antibodies with the ECL chemiluminescence system (Amersham Pharmacia Biotech).

Organ culture. A modified technique of organ culture using explants of human nasal turbinate mucosa was used (Jackson *et al.*, 1996; Read & Goodwin, 2001). Inferior turbinates derived from patients with non-allergic nasal obstruction were resected in all cases by the same surgeon. All donors gave informed consent and the research was approved by the South Sheffield Research Ethics Committee (96/260). Tissue was transported to the laboratory in Minimal Essential Medium (MEM) containing penicillin (50 000 units l⁻¹), streptomycin (50 mg l⁻¹) and gentamicin (50 mg l⁻¹) and dissected to produce 3–4 mm squares of mucosa, after removal of the anterior pole of the turbinate, which can be heavily populated by squamous epithelium. Tissue was incubated in antibiotic-containing MEM for a total of 4 h before being immersed in 20 ml antibiotic-free MEM for 1 h. Homogenates of explants treated in this way exhibited no antibiotic activity in bioassay (data not shown), suggesting that the methods used did not result in accumulation of antibiotics in tissue. A 3 cm Petri dish was placed within the perimeter of a 10 cm Petri dish, and Visking tubing (30/32; Scientific Instruments) placed across the inner dish to irrigate the explant with antibiotic-free MEM (7 ml) placed in the outer dish. Explants were then placed cut-surface downwards onto the filter paper across the centre of the inner dish and 1% molten agar (at 40 °C) was placed around the explant to hold its orientation. The agar set rapidly, and then tissue was bathed in 200 µl MEM until it was ready for inoculation with bacteria. In experiments to validate the invasion assay, explants were treated with and without cytochalasin D (1 µg ml⁻¹), which was added to all media bathing the tissue from arrival in the laboratory until completion of experiments.

Measurement of survival and penetration by *N. meningitidis*.

To measure survival of *Neisseria* spp. within the organ culture system, the MEM bathing the tissue was aspirated and 100 µl PBS containing a suspension of *Neisseria* spp. was placed onto the surface of the air-exposed explant. The whole was then transferred to an incubator at 37 °C in humidified 5% CO₂ and incubated over a period of 24 h. At intervals over the period of incubation, explants were carefully removed from the agar and transferred to PBS (6 ml) in a bijou container. Explants were vortexed and washed through three changes of PBS, then homogenized in a 1-shot cell disrupter (Warwick Systems, Warwick, UK). The total number of bacteria within each homogenate was then estimated by viable counting. A technique described previously (Read *et al.*, 1999) was used to estimate bacterial invasion of the tissue. Explants were immersed in 0.25% (w/v) sodium taurocholate (bile salts) (Sigma) for 30 s. Tissue was then immediately transferred to three changes of PBS in a universal container prior to homogenization in the 1-shot cell disrupter. This concentration of sodium taurocholate kills a suspension of 10⁷ *N. meningitidis* within 30 s (Read *et al.*, 1999). The mean bactericidal concentration (MBC) of sodium taurocholate for each strain under test was measured by a standard dilutional technique. Prior to homogenization, all tissue samples were weighed and the viable counts of the homogenates of both sodium-taurocholate-treated and untreated explants were expressed per mg tissue. Where required, an 'invasive fraction' was calculated by dividing the viable count of sodium-taurocholate-treated explants by the viable count of untreated explants. Output bacteria were checked by Gram staining. Uninfected control explants were included so that any contamination could be identified.

Statistical analysis. For multiple comparisons between strains, statistical analysis was conducted by two-way ANOVA. Residuals were checked for normality using the Anderson–Darling

Table 1. Strains used in this study

Species	NCTC no., acquisition no. or other identifier	Clonal group	Serogroup	Opa	PilC
<i>N. meningitidis</i>	K454	ET-5	B	+	+
<i>N. lactamica</i>	10617	NA	NA	—	+
<i>N. animalis</i>	10212 N462	NA	NA	—	—
<i>N. meningitidis</i>	z1275	A: subgroup I	A	—	+
<i>N. meningitidis</i>	z3524	A: subgroup III	A	+	+
<i>N. meningitidis</i>	z4421	A: subgroup IV-I	A	+	+
<i>N. meningitidis</i>	z4662	A4	B	+	+
<i>N. meningitidis</i>	z4242	ET-37	C	+	+
<i>N. meningitidis</i>	z4673	Lineage III	B	+	+
<i>N. meningitidis</i>	z3842	ET-5	B	+	+

NA, Not applicable.

test. To compare inter-subject variability, data from 40 patients were normalized by logarithmic transformation, prior to analysis of variance. Intra-class correlation between replicates was based on variance components and estimated by two-way ANOVA.

RESULTS

Validation of organ culture model

Results of preliminary experiments to refine and validate the model are shown in Fig. 1. Explants from a single human host were inoculated with 10-fold incremental doses of *N. meningitidis* from 10^2 c.f.u. to 10^7 c.f.u. (Fig. 1a). With inocula of 10^2 c.f.u. there was no recovery of *N. meningitidis* from explants homogenized at 4 h. With inocula of 10^3 and 10^4 c.f.u., small numbers of organisms

were recovered after 4 h incubation but none after 18 h incubation. Inocula of 10^6 and 10^7 c.f.u. resulted in recovery of organisms after 4 h incubation, with replication to higher counts by 18 h. With an inoculum of 10^5 c.f.u. there was recovery of bacteria after 4 h but numbers declined thereafter. After treatment with sodium taurocholate, bacteria were only recovered after both 4 h and 18 h incubation from explants inoculated with 10^7 c.f.u. (data not shown).

Fig. 1(b) demonstrates the yield of *N. meningitidis* (strain K454) in six experiments using human nasopharyngeal explants derived from six different human hosts. At an initial inoculum of $1.9 \pm 0.5 \times 10^7$ c.f.u. there was a gradual increase of the number of organisms recovered from untreated washed homogenates of ex-

Fig. 1. Growth of *N. meningitidis* strain K454 in nasopharyngeal explant mucosa. (a) Viable counts recovered from homogenized tissue following inoculation of 10^2 c.f.u. (∇), 10^3 c.f.u. ($\blacktriangle$), 10^4 c.f.u. ($\triangle$), 10^5 c.f.u. ($\blacksquare$), 10^6 c.f.u. ($\square$) and 10^7 c.f.u. ($\bullet$) after 4 h and 18 h incubation. (b) Viable counts of *N. meningitidis* strain K454 from nasal mucosa derived from six human donors over 18 h incubation, using tissue exposed ($\circ$) or not exposed ($\bullet$) to cytochalasin D. The dashed graphs show the number of bacteria recovered after sodium taurocholate treatment ($n=6$).

Table 2. Survival of representative strains of *N. meningitidis*, *N. lactamica* and *N. animalis* in human nasopharyngeal mucosa *in vitro*

Data are expressed as viable counts (mg explant tissue)⁻¹; means $\pm$ SEM, $n=9$ experiments. * $P=0.04$, *N. lactamica* versus *N. meningitidis*. ** $P=0.004$, *N. animalis* versus *N. meningitidis*; $P=0.024$, *N. animalis* versus *N. lactamica* (Mann-Whitney test). ns, Not significant.

	Inoculum (c.f.u.)	Bacteria recovered			
		4 h		18 h	
		Total	After sodium taurocholate treatment	Total	After sodium taurocholate treatment
<i>N. meningitidis</i>	$1.9 \pm 0.7 \times 10^7$	104.1 ± 23.6	0	1650.1 ± 1220.8	75.9 ± 28.3
<i>N. lactamica</i>	$1.3 \pm 0.6 \times 10^7$	133.4 ± 71.3 (ns)	0	$388.3 \pm 250.5^*$	0
<i>N. animalis</i>	$2.5 \pm 0.8 \times 10^7$	155.6 ± 57.5 (ns)	0	$3.8 \pm 2.0^{**}$	0

plant tissue over 18 h incubation. After treatment of explants with sodium taurocholate very few bacteria were recovered from explants incubated for 4 h (mean invasive fraction 0.024). However, this gradually increased and by 18 h a higher proportion of organisms was recovered after sodium taurocholate treatment, with a mean invasive fraction of 0.14.

Treatment of tissue with the f-actin polymerization inhibitor cytochalasin D reduced the yield of strain K454 from homogenized explants (Fig. 1b). Following sodium taurocholate treatment, no viable bacteria were recovered from explants exposed to cytochalasin, supporting the utility of sodium taurocholate in isolating bacteria internalized into the mucosa.

The MBC of sodium taurocholate for all strains used in the study was 0.03%. Thus, the concentration of sodium taurocholate used to kill extra-mucosal bacteria (0.25%) was more than eightfold higher than the MBC. To determine whether treatment with sodium taurocholate was killing all extra-mucosal neisseriae without killing those that had penetrated mucosa, explants infected with strain K454 for 24 h were washed, treated with sodium taurocholate and then washed a further three times prior to vigorous rolling over the surface of blood agar. No colonies of *Neisseria* grew over 24 h incubation ($n=3$). However, when the same explants were homogenized, viable counts of strain K454 were recovered from all three, at similar concentrations to those shown in Fig. 1(b) (data not shown). To demonstrate that sodium taurocholate did not penetrate the tissue, uninfected explants were treated with sodium taurocholate 0.25% for 30 s, washed, homogenized, and the homogenate was placed in wells cut into blood agar. No bactericidal activity was observed.

Comparison of *N. meningitidis*, *N. lactamica* and *N. animalis*

To determine whether this model discriminates among representative pathogenic and non-pathogenic *Neisseria* spp., explants were infected with *N. meningitidis* (strain

K454), and NCTC strains of *N. lactamica* and *N. animalis*. The strain of *N. lactamica* used in this study expressed PilC but not Opa, whilst the strain of *N. animalis* expressed neither (see Table 1). Data from nine repeated experiments each using tissue from a different human host, in which *Neisseria* recovery after 4 h and 18 h incubation was compared, are shown in Table 2. Explants weighed in the range 28–36 mg, with no significant difference observed between experimental limbs. There was no significant difference between the inoculum of organisms, and no significant difference between viable counts of homogenates of untreated explants after 4 h incubation. In contrast, the counts of *N. meningitidis* recovered from untreated explants by 18 h of incubation was significantly greater than those of *N. lactamica* and *N. animalis*. Very little change in the recovered counts of *N. lactamica* was seen over 18 h of infection; *N. animalis* was recovered at much lower counts from untreated explants after 18 h. In these experiments, there was no recovery of bacteria from explants incubated for 4 h and subsequently treated with sodium taurocholate; however, there was recovery of *N. meningitidis* from sodium-taurocholate-treated explants at 18 h, in contrast to *N. lactamica* and *N. animalis*, which were not recovered from sodium taurocholate-treated explants. Minor variations in inoculum into the model were observed but when the analysis was adjusted to account for this, all significant associations remained.

Comparison of clonally diverse isolates of *N. meningitidis*

Eight representative isolates of diverse clones of *N. meningitidis* were then grown to mid-exponential phase, washed, and each was inoculated onto a pair of explants derived from the same donor and incubated for 18 h, whereupon explants were homogenized with and without prior sodium taurocholate treatment. This required the use of 16 explants derived from the same donor on each experimental day. All strains were found to express

Table 3. Survival of representatives of clones of *N. meningitidis* in human nasopharyngeal mucosa *in vitro* over 18 h incubation

Data are expressed as viable counts (mg explant tissue)⁻¹; means $\pm$ SEM, $n=6$ experiments.

Clonal group	Serogroup	Inoculum (c.f.u. $\times 10^7$)	Bacteria recovered	
			Total ($\times 10^3$)	After sodium taurocholate treatment ($\times 10^3$)
A: subgroup I	A	2.1 $\pm$ 1.3	8.6 $\pm$ 13.0	0
A: subgroup III	A	1.5 $\pm$ 3.6	61.8 $\pm$ 133.4	0
A: subgroup IV-1	A	3.3 $\pm$ 3.1	2.8 $\pm$ 4.2	0
A4	B	1.7 $\pm$ 1.3	18.4 $\pm$ 38.5	0
ET-37	C	1.9 $\pm$ 2.0	13.3 $\pm$ 12.5	1.0 $\pm$ 1.9
Lineage III	B	0.8 $\pm$ 0.6	483.1 $\pm$ 999.6	8.1 $\pm$ 15.1
ET-5	B	0.7 $\pm$ 0.4	264.8 $\pm$ 121.1	5.6 $\pm$ 13.5
ET-37	B	3.2 $\pm$ 2.7	71.5 $\pm$ 57.5	4.5 $\pm$ 6.4

Opa and PilC, except *N. meningitidis* Z1275 (A: subgroup I) which did not express Opa. Data from experiments repeated six times with tissue from different donors are shown in Table 3. There was no significant difference between the inoculum size of the different isolates. Recovery of bacteria from explants incubated for 18 h only was measured. There was no significant difference between donor tissues in weight of tissue or recovery of bacteria from untreated explants. In contrast, isolates of A: subgroup I, A: subgroup III, A: subgroup IV-1 and A4 were consistently absent from sodium-taurocholate-treated explants, whereas strains of ET-37, lineage III and ET-5 were consistently recovered within such mucosa of all six donor tissues used. It was noted that the ET-5 strain was recovered in higher counts than was seen in experiments described in Table 2 (in which another ET-5 strain, K454) was used. This was likely due to wide inter-donor variability (see below).

Variation between tissues derived from different human subjects

Explants from 40 different human donors were cultured identically, infected with strain K454 and incubated for 18 h. After this period of time triplicate explants from each donor were treated with sodium taurocholate or left untreated and then homogenized prior to viable counting. Amongst the 40 donors, six were smokers, there were 19 females, 21 males, and 16 of the explants were resected during the winter months (November to March). The mean inoculum was $3.9 \pm 1.56 \times 10^7$ (range 2×10^6 – 9×10^7). The mean recovery of *N. meningitidis* from untreated explants after 4 h of infection was 160.2 ± 49.7 c.f.u. (mg tissue)⁻¹ (range 2.6–1578.95), rising to 47717 ± 25190 c.f.u. mg⁻¹ (range 30–937500 c.f.u. mg⁻¹) after 18 h incubation. The recovery of bacteria from sodium-taurocholate-treated explants after 4 h incubation was 2.35 ± 0.88 c.f.u. mg⁻¹ (range 0–30.3 c.f.u. mg⁻¹) rising to 4093 ± 3026 c.f.u. mg⁻¹

Fig. 2. Box-and-whisker plots of *N. meningitidis* recovery from identically harvested explants derived from 40 human adult donors which were experimentally infected with strain K454 *N. meningitidis*. Plots are shown for total bacteria recovered from washed explants ('total') and (as an estimate of invasion) from sodium-taurocholate-treated explants ('protected'). Outliers are plotted with asterisks.

(range 3–120000 c.f.u. mg⁻¹) after 18 h incubation. The mean invasive fraction at 4 h was 0.021 ± 0.009 at 4 h, and 0.118 ± 0.027 at 18 h. There was no effect of smoking, season or sex on the recovery of bacteria from explants, by analysis of variance.

Variance of recovery of bacteria from explants is illustrated in Fig. 2. Analysis of triplicate data derived from individual donor tissues showed that the methods were highly reliable, with an intraclass correlation at 4 h of 0.988, and at 18 h of 0.968 (of c.f.u. per mg of untreated explants). When recovered viable counts were adjusted for minor variation of the inoculum size, the

coefficient of variation of recovered viable counts from untreated explants was 1335% after 4 h incubation, and 77% after 18 h incubation.

DISCUSSION

An organ culture model was used to measure the survival of *Neisseria* spp. on and within human nasopharyngeal mucosa, and the results indicate that the model is able to discriminate between *N. meningitidis*, and representative strains of *N. lactamica* and *N. animalis*. There were clear differences in survival within the mucosa of clonally diverse strains of *N. meningitidis*. Survival of *N. meningitidis* within nasopharyngeal tissue varied widely between mucosae derived from different human hosts, at least in the model used in this study. The experimental period of 18 h was sufficiently long to permit emergence of any variants with invasive potential.

Overall survival of *Neisseria* within the mucosa was measured by homogenizing washed explants infected with organisms over variable lengths of time. The use of sodium taurocholate to kill extramucosal bacteria is reasonable; compared with other bile salts, sodium taurocholate has a low pK_a and requires specialized active transport for efficient penetration of epithelial cells (Shiau, 1987). Invasion of nasopharyngeal explants by *N. meningitidis* is an active process of parasite-directed endocytosis (Stephens *et al.*, 1983; Read *et al.*, 1995). During this process, bacteria are enveloped by microvillous extensions from the cell surface, a process requiring host cell cytoskeletal rearrangement, which in turn requires polymerization of actin filaments. Failure to recover any bacteria from cytochalasin D-exposed explants after treatment with sodium taurocholate (in contrast to explants not exposed to cytochalasin D) suggests that sodium taurocholate kills all bacteria which have not invaded the mucosa as a result of endocytosis, or some other process requiring an active host response. We demonstrated no bactericidal activity of homogenates of sodium-taurocholate-treated explants, indicating that sodium taurocholate did not penetrate this tissue. We also demonstrated absence of meningococci on the surface of sodium-taurocholate-treated infected explants and were only able to retrieve meningococci from such explants once they had been homogenized. This suggests that sodium taurocholate treatment kills extra-mucosal bacteria, but does not kill bacteria within a protected site deep within the mucosa. Sodium taurocholate kills bacteria rapidly, in contrast with gentamicin (which is often used for similar assays of invasion), which requires prolonged periods of incubation for bactericidal activity.

We found that high inocula (10^7 c.f.u.) of *N. meningitidis* were required for consistent meningococcal survival in this model. Inocula of 10^5 c.f.u. or below failed to thrive, whilst an inoculum of 10^6 c.f.u. did not result in survival of sufficiently high numbers within the tissue. The natural inoculum during droplet transmission between humans cannot be accurately measured, but is probably

orders of magnitude below that used in this model. On the other hand, the surface area of mucosa available for epithelial contact by droplet-associated *Neisseria* during natural transmission is very much higher than that available in this experimental method.

The failure of *N. animalis* to thrive in this experimental model is consistent with its habitat – it has never been isolated from humans but does colonize a range of small mammals including guinea pigs (Morse & Genco, 1998). *N. lactamica* survived in relatively low numbers but, in contrast to *N. meningitidis*, could not be recovered from sodium-taurocholate-treated explants. The behaviour of single strains should not be overinterpreted but these data suggest that survival of *Neisseria* spp. within nasal mucosa may correlate with pathogenicity. However, the survival of diverse isolates of *N. meningitidis* was not uniform in these experiments. Meningococci belonging to the subgroups of serogroup A could not be recovered from an intramucosal site, whereas the representative isolates of the ET-37 complex, lineage III and ET-5 complex did invade and grow intramucosally. These observations are intriguing, given the diverse epidemiologies of different meningococcal lineages. Strains belonging to the subgroups of serogroup A cause large-scale epidemics and pandemics in China and Africa, but have caused virtually no disease in Western Europe and North America since the Second World War (Schwartz *et al.*, 1989). By contrast, ET-37 complex meningococci cause up to 40% of meningococcal disease in European and American countries and are commonly associated with institutional outbreaks of serogroup C disease. Members of the ET-5 complex were responsible for the spread of hyperendemic meningococcal disease during the 1970s and 1980s in Western Europe, but were largely replaced as a major cause of meningococcal disease in many countries by organisms belonging to lineage III. The reasons for these diverse epidemiologies are not completely understood, but it is thought that ET-5 complex and lineage III organisms, for example, may have prolonged carriage compared to meningococci belonging to the subgroups of serogroup A. The $t_{1/2}$ of carriage of serogroup A meningococci is 1 month, whereas the $t_{1/2}$ of carriage of meningococci belonging to other serogroups is about 3 months (Blakebrough *et al.*, 1982).

Our data suggest that differences in the potential of genetically diverse meningococci to penetrate human nasopharyngeal mucosa can be measured in this model system, potentially yielding information valuable to the understanding of meningococcal epidemiology and pathogenesis. This diversity of invasion and intramucosal growth is likely due to a combination of several meningococcal cellular components, including outer-membrane proteins, capsule, IgA protease and pili (Nassif *et al.*, 1999). Although Opa and PilC have been clearly shown to influence successful attachment to epithelial cell monolayers and organ culture mucosa (Virji *et al.*, 1992, 1993; Rayner *et al.*, 1995) the data presented here suggest that other meningococcal determinants are also required for survival within the mucosa.

More experimentation using organ culture systems is required to address these issues, perhaps employing isogenic meningococcal constructs. The availability of whole genome sequences for comparison of serogroup A subgroup (IV) (Parkhill *et al.*, 2000) with a member of the ET-5 complex (Tettelin *et al.*, 2000) will open up new possibilities for developing further these observations in this *in vitro* model.

One remarkable finding was the very wide variation in the recovery of meningococci from nasopharyngeal explants derived from different humans, which was initially apparent in experiments investigating differences between *Neisseria* species and clonal groups (Tables 2 and 3). This was then formally tested using tissue from 40 human donors (Fig. 2). Experiments were conducted in triplicate and there was very little variation between explants derived from an individual host. However, the coefficient of variation across the group of 40 individuals was 1335 % after 4 h and 77 % after 24 h incubation. This is dramatic inter-subject variability – in comparison, the coefficient of variation of the human haematocrit is 9 %, and that of the height of 17-year-old males is only 3.4 % (Lentner, 1984). As the experimental methodology was uniform across the 40 individuals studied, the variation that was observed was likely due to genetic or environmental influences upon the host tissue. All of the individuals who donated tissue in this survey were suffering from non-allergic nasal obstruction, mainly due to septal deviation. None were receiving intranasal topical steroids, though clearly we cannot exclude the influence of disease comorbidities secondary to the upper respiratory tract obstruction. Other possible environmental influences that could explain this variation include recent respiratory tract infection, though there was no correlation with season. It is unlikely that anti-meningococcal antibody present in the mucosae was responsible for the variation observed, as the tissue employed is not lymphoid, and we have not detected antibody to common viruses (e.g. influenza) in tissue homogenates (Read *et al.*, 1999).

Epidemiologically, there is support for the notion of a genetic influence on successful colonization and invasion. Within communities, nasopharyngeal colonization by *N. meningitidis* is only rarely complicated by invasive disease, even amongst those who do not possess serogroup- or serotype-specific antibodies (Goldschneider *et al.*, 1969). Likewise, individuals differ in the characteristics of nasopharyngeal carriage of meningococci. Rake (1934) followed the nasopharyngeal carriage of meningococci of groups of individuals over a period of 2 years. This included one group in a confined meningococcal laboratory amongst whom 50 % were never colonized despite the fact that they are likely to have been engaged regularly in oral pipetting of meningococcal cultures. Amongst those who became colonized, there were three patterns: some were transient, some were intermittent and others were chronic carriers of the meningococcus. This pattern was also observed in young children in a nursery school environment (Rake, 1934).

We conclude that the distinctive colonization and disease potential of *Neisseria* spp. may be partially a consequence of their ability to invade and survive within human nasopharyngeal mucosa, but that this is influenced greatly by host or environmental factors.

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