

INTERNATIONAL *BURKHOLDERIA CEPACIA* WORKING GROUP

Minutes of the Fifth Meeting

Miami, Florida
March 14 - 15, 1998

(prepared by John LiPuma)

The International *Burkholderia cepacia* Working Group (IBCWG) met for the fifth time in Miami, Florida on March 14-15, 1998. The meeting was co-chaired by Jane L. Burns, John LiPuma, and David Speert. In attendance were 34 scientists and clinicians from the United States, Canada, the United Kingdom, Belgium, Germany, France, Italy and Australia (appendix). The meeting was hosted by Zeneca Pharma (Canada) with additional support provided by the national cystic fibrosis charitable organizations.

As with the previous meetings the overall objective of this gathering was to advance our understanding of *B. cepacia* infection/colonization in persons with cystic fibrosis through collegial exchange of information and promotion of coordinated approaches to research.

A number of reports and short presentations were made to the entire group before participants broke into sub-groups for more in-depth discussion.

REPORTS

Update on taxonomy of *B. cepacia* complex

Peter Vandamme

Taxonomic studies of putative *B. cepacia* isolates of human (CF & non-CF) and environmental origin from 1993 to 1995 were summarized. These studies employed whole-cell protein electrophoresis, whole-cell fatty acid analysis, DNA-DNA and DNA-rRNA hybridization experiments, DNA base ratio analyses and biochemical analysis.

Conclusions:

1. *B. cepacia* comprises at least five distinct genotypic species, referred to as genomovars (Ursing et al., 1995).
2. Genomovar V was identified as *Burkholderia vietnamiensis*, a recently described species which fixes nitrogen and is associated with rice roots (Gillis et al., 1995).
3. Genomovar II could be differentiated from the other genomovars by means of classical biochemical tests. Therefore, a novel name, *Burkholderia multivorans*, has been proposed.
4. Genomovars I, III, and IV could not be differentiated by means of classical biochemical tests and therefore, no nomenclatural modifications were proposed. These species are presently referred to as *B. cepacia* genomovar I, *B. cepacia* genomovar III, and *B. cepacia* genomovar IV.
5. All five genomovars occur in CF patients.
6. The term "*B. cepacia* complex" was proposed for the entire group of five genomovars as proposed during the 1997 Victoria IBCWG meeting. *B. gladioli* therefore does not belong to the *B. cepacia* complex.

(Reference: Vandamme et al. 1997. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int. J. Syst. Bacteriol. **47**:1188-1200).

Subsequent identification analyses of well-characterized strains:

1. The majority of CF isolates is genomovar III (about 50%), followed by *B. multivorans* (about 24%), and *B. vietnamiensis* (about 15%). Genomovars I, IV, and *B. gladioli* represent minor fractions.
2. Most of the environmental (except hospital) strains are genomovar I (about 50%), followed by *B. vietnamiensis* (about 32%), and *B. gladioli* (about 16%). Genomovars III, and IV, and *B. multivorans* represent minor fractions or have not been identified.
3. Many putative *B. cepacia* strains remain unidentified.
4. Major epidemic strains (ET 12, Manchester & Newcastle epidemics) are genomovar III.
5. Cases of "cepacia syndrome" are genomovar III.
6. Some genomovar III strains carry the cable pilus.
7. Many but not all genomovar III strains carry the BCESM marker
8. Cable pilus and BCESM marker seem to occur outside genomovar III as well, although the identification is sometimes doubtful and should be confirmed by DNA-DNA hybridization experiments.
9. Glasgow epidemic is genomovar II.
10. All (n= 7) isolates with biotechnological potential were identified as *B. vietnamiensis*.

What's new?

Based on whole-cell protein electrophoretic similarity & DNA-DNA hybridization experiments

| <u>Group</u> | <u>Phylogenetic allocation</u> | <u>Source</u> |
|--------------|---|---|
| 1 | Novel sp. <i>Ralstonia</i> | Environm. |
| 2 | Novel sp. <i>Ralstonia</i> | Environm. & human, non-CF |
| 4 | Novel sp. <i>Ralstonia</i> | Environm. |
| 5 | Novel sp. <i>Ralstonia</i> | Environm. |
| 6 | Novel sp. <i>Ralstonia</i> | Environm. & human, non-CF |
| 7 | Novel sp. <i>Bordetella/Alcaligenes</i> | Human, non-CF |
| 9 | Novel sp.? <i>Bordetella/Alcaligenes</i> | Human, non-CF |
| 10 | Novel sp.? <i>Bordetella/Alcaligenes</i> | Environm. & human, non-CF |
| 11 | Novel sp.? <i>Burkholderia</i> | Environm. & human, non-CF |
| 12 | Novel sp.? <i>Ralstonia</i> | CF |
| 13 | Novel sp.? <i>B. cepacia</i> complex | CF & environm. |
| 14 | Novel sp.? <i>Ralstonia</i> | Environm. & human, non-CF & transplant pts |
| 15 | Novel sp.?? <i>Ralstonia</i> | Environm. & human, non-CF & CF |
| 16 | Novel sp.?? <i>Ralstonia</i> | Human, non-CF & CF |
| 17 | Novel sp.?? <i>Ralstonia</i> | Environm. & CF |
| 19 | Novel sp.?? <i>Ralstonia</i> | CF |
| 20 | Novel sp.? <i>Burkholderia</i> | Environm. |
| 21 | Novel sp.?? ? | Environm. |
| 22 | Novel sp.?? <i>Burkholderia</i> (Bcc?) | Environm. & CF |
| 23 | Novel sp.? <i>Ralstonia</i> | CF |
| 25 | Novel sp.? <i>Ralstonia</i> | CF |

Species

Source

| | |
|---------------------------------|--|
| <i>B. andropogonis</i> | Plant pathogen (sorghum, velvet bean) |
| <i>B. caryophylli</i> | Plant pathogen (carnation) |
| <i>B. cepacia</i> genomovar I | Plant pathogen (onion, <i>Lycopersicon</i>) soil, water, human (non-CF & CF) |
| <i>B. cepacia</i> genomovar III | Hospital environment, human (non-CF & CF) (CF: Australia, Austria, Belgium, Canada, Ireland, Sweden, Switzerland, UK, USA, [Italy??]) |
| <i>B. cepacia</i> genomovar IV | Hospital environment, human (non-CF & CF) |
| <i>B. gladioli</i> | Plant pathogen (onion, <i>Gladiolus</i> , <i>Iris</i>), CF, and cases of food poisoning |
| <i>B. glathei</i> | Soil |
| <i>B. glumae</i> | Plant pathogen (rice) |
| <i>B. mallei</i> | Animals (horses, donkeys) |
| <i>B. multivorans</i> | Plant associated soil, human (non-CF & CF) |
| " <i>B. norimbergensis</i> " | Water |
| <i>B. plantarii</i> | Plant pathogen (rice) or plant associated (<i>Vanda</i> sp.) |
| <i>B. pseudomallei</i> | Soil, water, & cases of melioidosis in humans and animals |
| <i>B. pyrrocinia</i> | Soil |
| " <i>B. thailandensis</i> " | Environmental |
| <i>B. vietnamiensis</i> | Soil, nitrogen fixer in rice & human, non-CF & CF |

B. gladioli = *B. cocovenenans* (toxin producer)

B. plantarii = *B. vandii*

Coenye et al. (submitted for publication)

What is the source of genomovar III strains?

1. CF patients are colonized with a variety of different clones belonging to genomovar III. Novel *B. cepacia* positive patients emerge regularly.
2. Genomovar I, III, and IV are difficult to differentiate.
3. Modified protein electrophoretic analyses do not reveal any differences at all between genomovar I, III, and IV strains, but allow to differentiate *B. multivorans*, *B. vietnamiensis*, *B. gladioli*, and other burkholderias.
4. Is there is genomic process that changes environmental genomovar I strains while they are in the CF host into genomovar III strains? This would have to be a regularly occurring phenomenon that would cause a decrease in overall DNA-DNA reassociation of some 30%; the results of the rearrangements are always the same: the strains are protein electrophoretically very similar and share high levels of DNA-DNA reassociation??? This is taxonomic heresy...
5. Alternatively, have these modifications occurred only once or a few times, which resulted in rapidly evolving clones spread via CF patients?

Update on US repository

John LiPuma

The CFF/AUHS *B. cepacia* Research Laboratory and Repository was established in Spring 1997 to assist U.S. CF treatment centers in identification and genotyping of *B. cepacia*. A polyphasic approach to species identification using selective media, conventional biochemical assays and PCR-based assays is employed for all isolates. The first isolate was accepted June 10, 1997. In the first 9 months of operation 357 isolates have been received from 261 individuals from 71 treatment centers. Of the 343 that have been analyzed, 267 were identified as *B. cepacia* by the referring labs and the remaining 76 were referred as "unknown" or as a species other than *B. cepacia*. Among the 267 identified as *B. cepacia* by referring lab, 229 were confirmed as *B. cepacia* and 38 (14%) were found to NOT fit criteria for *B. cepacia* complex. The majority of the latter were sent to Peter Vandamme for taxonomic evaluation; most were "unidentified" by that analysis, although 9 were *B. gladioli* and 5 were *P. aeruginosa*. Among the 76 isolates referred to the lab as something other than *B. cepacia*, 22 (29%) were, in fact, found to be *B. cepacia*. The laboratory has also provided genotyping, using both PCR-ribotyping and RAPD typing, of 112 isolates from 17 centers.

Update on Canadian repository

Esh Mahenthiralingam

Epidemiology and clinical outcome of infection with *B. cepacia* complex strain among patients with CF in British Columbia, Canada: A retrospective study investigating all CF patients from whom *B. cepacia* was cultured since 1981 in Vancouver, BC, was presented. A comparison was performed of clinical outcome associated with colonization with *B. cepacia* genomovar III strains and *B. multivorans*. Since 1981 a total of 62 colonized CF patients have been treated; 56 were examined in detail. Of these, 38 were colonized with genomovar III strains of RAPD types 1, 2, 4 and 6. Each of these types encode the *B. cepacia* epidemic strain marker; RAPD type 2 strains also encode the cable pilus gene (the ET12 lineage). Of the remaining colonized patients, 18 were infected with unique *B. multivorans* strains; BCESM-negative strains of undetermined genomovar were cultured from 6 patients. Four epidemiological characteristics were striking in the patients colonized with BCESM positive genomovar III strains: (i) the highest mortality (20/38 died), (ii) patient-to-patient spread, (iii) replacement of the *B. multivorans* infection with BCESM positive strains in 6 patients, 2 of whom subsequently died, (iv) chronic infection in comparison with *B. multivorans* where several patients were only transiently colonized. Preliminary studies of lung function and body mass characteristics also suggested that genomovar III strains are associated with a more rapid decline in clinical condition when compared with *B. multivorans*. These data indicate that at least in British Columbia colonization with strains of genomovar III which possess the BCESM DNA pose the greatest risk to the health of patients with CF. A table summarizing these data is shown below.

Table: A comparison of *B. cepacia* BCESM positive genomovar III and *B. multivorans* infection among CF patients in British Columbia.

| <u>Characteristic</u> | <u><i>B. cepacia</i> BCESM (+)</u> | <u><i>B. multivorans</i></u> |
|--|------------------------------------|---|
| No. of patients | 38 | 18 |
| % mortality | 52 | 0 (11 if associated with BCESM+ strain) |
| Mean age first isolate frozen in collection | 19.3 | 13.1 |

Table (continued)

| <u>Characteristic</u> | <u><i>B. cepacia</i> BCESM (+)</u> | <u><i>B. multivorans</i></u> |
|--|------------------------------------|-----------------------------------|
| % Mortality in patients less than 18 yrs old | 43 | 0 |
| % patients infected for greater than 1 year | 81 | 33 |
| Colonization of multiple patients | Yes | No (one sib pair - but transient) |
| Ability to replace <i>B. multivorans</i> infection | Yes | No |
| Ability to replace genomovar III BCESM (+) infection | Yes | No |

***B. cepacia* in Europe**

John Govan, Adolf Bauernfeind

John Govan announced the presence of the IBCWG microbial "mascot" - a *B. cepacia* of uncertain genomovar from Opreyland Hotel "soil" in Nashville. He also emphasized the value of old world "collections" as well as newer North American repositories. The pro-active collecting of isolates in Edinburgh from the 1980's (now exceeding 1200 isolates) provides valuable material when problems occur. E.g., documented clinical data and the first UK isolates of ET12 lineage and 1986 genomovar III cepacia syndrome isolate with no evidence of epidemic markers and no transmission to the patient's CF sibling, plus the value of banks of longitudinal isolates and sera from patients. The need for the IBCWG to address new cepacia guidelines and new twists to the problem of transmission was described. E.g. (i) cepacia-negative CF patient with a cepacia-positive CF brother; (ii) cepacia transfer from CF patient to non-CF mother with subsequent bronchiectasis (Liverpool); (iii) superinfection of four *B. cepacia*-positive individuals with ET12 lineage leading to 3/4 rapid fatalities (Liverpool). (Does this lineage require different infection control?); (iv) fatal colonization and possible cross-infection when ITU-treated CF patient (traffic accident) shares unit with non CF patients (Dundee). CF patients and one non CF died; non CF isolate belongs to genomovar III and similar isolates cultured from ITU environment. CF patient's strain has different PFGE pattern from non CF patients; (v) JRWG's Zimbabwean PhD student shares "international" apartment with Swedish microbiology student who has CF brother (Edinburgh). Low risk but much concern on the possibilities of indirect transfer during brother's visit.

Adolf Bauernfeind discussed Burkholderia spp. in Germany. The overall prevalence of *B. cepacia* complex strains in CF patients in Germany in 1997 was 1.9% of 1654 patients. This number is probably too low as sputum specimens of only about one fifth of the patients were cultured on *B. cepacia* selective media. The yield of *B. cepacia* on unselective media was only 1.5% in comparison with 3.6% on selective media. In the Munich area where a *B. cepacia* selective medium has been used since 1987 the 10 years' prevalence was $37/520 = 7.1\%$ of the patients. The distribution of the *B. cepacia* genomovars among the Munich isolates will be analyzed retrospectively.

SHORT PRESENTATIONS

Patterns of *B. cepacia* colonization: association with mortality and lung function decline"

Liz Tullis/Mary Corey

A review of the experience in Toronto with *B. cepacia* was described. Salient points of interest included that for unclear reasons the incidence and prevalence of *P. aeruginosa* colonization have decreased as *B. cepacia* colonization rates have increased. Also of note is that the ages of patients at the time of acquisition of *P. aeruginosa* and *B. cepacia* has increased from the 1970s (3 yrs and 16 yrs, respectively) to the 1980s (5 yrs and > 18 yrs, respectively). Among *B. cepacia* colonized individuals, the clinical course seems worse for those who were previously colonized with *P. aeruginosa* than for those concurrently colonized with both *B. cepacia* and *P. aeruginosa*.

***Burkholderia cepacia* in the United Kingdom**

Stuart Elborn

A survey conducted in early 1998 of UK CF Centers representing almost 4,000 patients demonstrated a prevalence of *B. cepacia* infection ranging from 0% in 5 centers to over 30% in 2 centers. The prevalence in pediatric centers (median < 5%) was substantially lower than that seen in adult centers (median 10- 20%). There was a striking gradient from the southeast of the United Kingdom to the northwest with the highest prevalence centers being Manchester, Belfast, Glasgow, Edinburgh and Liverpool. In centers where segregation has been in place for 4 or more years (including those with high prevalence) less than 1 patient per year is becoming infected with *B. cepacia*. All UK centers surveyed segregate patients with *B. cepacia* from other groups for inpatient and outpatient care. The clinical outcomes of patients affected with *B. cepacia* compared to those with *P. aeruginosa* or neither infection was compared in patients from Belfast and demonstrated a mean change/year (over 5 years) in FEV1 of 1.6 (1) percent in those growing neither *P. aeruginosa* or *B. cepacia* and -4 (7) percent in those infected with *P. aeruginosa* and 5.4 (5) percent in those infected by *B. cepacia*. Reduced survival was noted in patients infected with *B. cepacia*. Data was also presented indicating that patients infected by *B. cepacia* had some evidence of increased inflammation with higher levels of neutrophil elastase alpha-1 anti-protease complex. Current approaches to antibiotics suggested that when pulmonary exacerbations of *B. cepacia* were treated with antibiotics to which the organism showed in vitro sensitivity, a better improvement in pulmonary function was seen compared to pan-resistant organisms treated empirically. However the exact role of multiple antibiotic testing such as combination or synergy testing is unclear.

Distribution of *B. cepacia* in the CF lung

Uma Sajjan and Janet Forstner

Immunolocalization of *B. cepacia* in peripheral regions of a CF lung (Toronto transplant patient) showed bacteria distributed over bronchiolar epithelia, between surface cells, in deeper submucosal layers, luminal exudates and alveolar septa. A specific anti-cable adhesin antibody identified the *B. cepacia* as belonging to the hyper-transmissible ET, Cbl-piliated, GV III strain. Neighboring lung sections were also incubated in vitro with exogenously added FITC-labelled *B. cepacia* isolates of all five genomovars. Only GV III Cbl-positive strains bound to lung sections. Bacteria were noted over airway epithelia, luminal exudates, alveolar septa and regions of squamous metaplasia. Binding was inhibited by the anti-cable adhesin antibody. Thus Cbl-piliated *B. cepacia* can adhere to and colonize peripheral lung tissue of susceptible CF patients.

High dose intravenous immunoglobulins for the treatment of "*B. cepacia* syndrome"

Giantonio Cazzola

Host immune response and inflammatory damage are important in the pathogenesis of *B. cepacia* infection; *B. cepacia* invokes high TNF induction and increased secretion of other cytokines and acute phase proteins. Therefore, anticytokine therapy may prove worthwhile in treatment of *B. cepacia* infection when antibiotic and steroid therapy is ineffective. Recently, IVIG preparations have been shown to regulate disturbed cytokine activity and anecdotal reports indicate that IVIG may be helpful in some cases of cepacia syndrome. In our center IVIG therapy (400 mg/kg/day for 3 days) was effective in a 14 year old girl with cepacia syndrome which had persisted after two weeks of antimicrobial and steroid therapy. Nevertheless, one year later IVIG did not modify the clinical course when the same patient exhibited persistent fever. The patient eventually responded to corticosteroid therapy. Regarding the use of IVIG in cepacia syndrome we conclude: (a) a more precise definition of cepacia syndrome is needed; (b) an immunopathological "rationale" exists for use of IVIG; (c) there are conflicting results in anecdotal reports; and (d) there is a need for further clinical trials before a therapeutic role can be established.

Inhibition by dextran of *B. cepacia* adherence to epithelial cells

Cheng-Hsun Chiu

B. cepacia appears to establish infection by adhering to respiratory tract epithelial cells and then contaminating the lower respiratory tract. To date no effective antiadhesive strategy has been devised for preventing *B. cepacia* infection. This study was done to evaluate the potential for preventing adhesion with dextran. Dextran (4,000 MW) at a concentration of 20 mg/ml significantly inhibited the adherence to A549 pneumocytes of all *B. cepacia* strains, except *cbIA*-positive epidemic strains C5424 and FC475. Morphologically, these two strains show microcolony formation, when they bind to A549 cells, while other strains show single cell binding predominantly. Dextran at a dose of 40 mg/ml or greater is needed to achieve the same level of inhibition in binding as well as microcolony formation of the two cable pilus-expressing strains. The inhibitory effect of dextran is reversible; if either bacteria or epithelial cells were pretreated with dextran, then washed before coincubation, the inhibitory effect was largely abrogated. Furthermore, sheared bacterial supernatant of C5424 was inhibitory, while inhibition was not reversed if the epithelial cells were pretreated and then either washed or unwashed before the treatment of the bacteria. The results suggest that cable pili are involved in adherence as well as microcolony formation of the epidemic *B. cepacia* strains. Dextran is an inexpensive and nontoxic agent and may be used in the future to prevent colonization and infection with *B. cepacia*.

Invasion and survival of *B. cepacia* in amoebae and macrophages

Miguel Valvano

Data were presented that demonstrated the ingestion of *B. cepacia* by amoebae into large vacuoles. Internalization into macrophages was demonstrated by acridine orange staining and use of crystal violet to kill extracellular organisms. These data suggest that *B. cepacia* resembles other facultative intracellular pathogens.

Genetic diversity of French *B. cepacia* strains

Edouard Bingen

Data are derived from the Observatoire National de *B. cepacia* 1996 (G. Chabanon, Y. Michel-Briand, E. Bingen) which includes information from 70 AFLM accredited CF care centers (n= 3500 patients). The prevalence of colonization with *B. cepacia* is 3%. There are marked regional difference in *B. cepacia* colonization. The mean colonization age is 12 years (range < 1-32 years). Ribotyping was used to study 124 isolates recovered from 124 patients with CF from 13 CF clinics in France. Twenty eight ribotypes were identified among the 124 CF isolates in which:

- * one to 29 isolates shared the same pattern
- * 17 isolates possessed unique ribotypes

- * 5 ribotypes were shared by 2 patients
- * 6 ribotypes were shared by 3 to 29 patients
- * Some predominant strain types were widespread in French CF clinics

The prevalence of BCESM was 71% among CF epidemic types and 24% among CF unique type. In conclusion, 5 strain types infected 6 CF patients and 2 strain types were detected in different regions. BCESM was associated with highly transmissible lineages. MICs were determined for 66 isolates with different ribotypes and antibiotic susceptibility patterns. Among β -lactam agents temocillin and meropenem displayed the most important inhibitory activities with 82 and 67% of susceptible strains and MIC 90s of 32 and 8 Hg/ml respectively

Genomic and phenotypic characterization of clinical and environmental isolates of *B. cepacia*

Tom Lessie

A review of the genetics of *B. cepacia* complex bacteria was presented. These bacteria have a large genome ranging from 5 to 9 Mb. Clinical and environmental strains cannot be differentiated based on the size of the genome nor on the number of replicons, although *B. multivorans* tend to have only two chromosomes. Strains from clinical and environmental sources are also difficult to differentiate phenotypically. For example, strains belonging to all 5 genomovars produce N-acyl homoserine lactones involved in quorum sensing.

Quorum sensing in *B. cepacia*

Pam Sokol

luxR/luxI homologues involved in quorum sensing in *B. cepacia* have been identified and designated *cepR/cepI*. These genes have significant homology to quorum sensing genes from *P. aeruginosa* and *Ralstonia solanacearum*. A transposon insertion in *cepR* results in a mutant which produces increased amounts of ornibactin and decreased amounts of lipase and protease. Therefore quorum sensing appears to be involved in the positive regulation of protease and lipase and the negative regulation of ornibactin synthesis.

Biochemical (phenotypic) identification of the *B. cepacia* complex

Deb Henry

Biochemical tests that could be used to identify the members of the *B. cepacia* complex and to speciate *B. gladioli* and *Ralstonia* species (formerly *B. pickettii*) were developed. The most useful tests were oxidase, ONPG, lysine decarboxylase, sucrose and adonitol (ribitol) oxidation, nitrate reduction, gelatin liquefaction and growth at 42C. Copies of tables were distributed, and can be obtained by emailing/faxing Deb Henry at dhenry@intergate.bc.ca; 604 875-2226.

Use of the *recA* gene to speciate *B. cepacia* by PCR

Esh Mahenthalingam

There is an urgent need to develop genomovar specific molecular diagnostic probes for the *B. cepacia* complex. We examined genes shared by all members of the *B. cepacia* complex, the 16S RNA gene and the *recA* gene, in order to identify if sufficient DNA sequence variation (polymorphism) is present to enable discrimination among genomovars. Preliminary studies demonstrate that sufficient sequence polymorphism is present within the *recA* gene to enable strains of each currently designated genomovar to be distinguished. Oligonucleotide

primers for PCR were designed to amplify *recA* from 30 strains representative of all five genomovars of the *B. cepacia* complex. Sequence variation within the 1040 bp amplified product was detected by restriction fragment length polymorphisms (RFLP) resolved by agarose gel electrophoresis. Genomovar specific RFLP patterns were observed within the 30 strains tested and suggested that the approach may be useful as a classification scheme. When polymorphisms within the 16S rRNA gene were examined, we were only able to identify variation sufficient to distinguish *B. vietnamiensis* and *B. multivorans* from each other, and separate these from genomovar I, III and IV. However, genomovar I, III and IV strains could not be separated by RFLP analysis of the 16S rRNA gene. We are beginning sequence analysis of the *recA* gene types within the *B. cepacia* complex in order to design species specific probes.

rRNA-based PCR detection of *B. cepacia* complex

John LiPuma & Paul Whitby

DNA encoding rRNA provides a useful target for development of species- and genomovar-specific PCR assays. Dr. Whitby discussed work with previously described primers based on 16S and 23S rRNA sequences have proved useful in detecting *B. cepacia* from sputum in one CF center; some individuals were found to be PCR-positive and culture-negative. More recent work has focused on sequencing rRNA operons from strains belonging to all five *B. cepacia* complex genomovars. Dr. LiPuma described the development of PCR assays, based on 16S sequence data, that are sensitive and specific for detection of *B. multivorans* and *B. vietnamiensis*. Primers based on 16S and 23S sequences specific for the remaining genomovars are being evaluated.

Molecular diagnosis of *B. cepacia* complex - PCR positive/culture negative experiences

John Moore

Employing a specific and sensitive PCR assay, we can demonstrate the presence of *B. cepacia* directly from sputum which are culture-negative by conventional bacteriological approaches. We postulate that prior to a rapid decline in pulmonary function due to *B. cepacia*, this organism may initially exist at very low numbers which are non-detectable by conventional culture. Our studies probably demonstrate the presence of low numbers of *B. cepacia* in the CF lung, similar to the PCR detection of low numbers of *Pseudomonas aeruginosa*. These data therefore requires further investigation to ascertain their clinical significance in order to direct appropriate cross-infection control procedures.

SUBGROUP DISCUSSIONS

Virulence Subgroup

Discussion began with the question: does the *Burkholderia cepacia* complex contain pathogens? It was felt that pathogenicity does not necessarily require demonstration of sepsis. Virulence in CF was based on: (i) risk of rapid, unexpected decline including pneumonia with or without bacteremia, (ii) transmission from patient to patient, (iii) *B. cepacia* as an independent factor for clinical decline and reduced life expectancy, (iv) apparent independence from other CF pathogens, and (v) evidence of causing pneumonia in CF mice, but not in control mice. The list of putative virulence factors that was constructed at the Victoria meeting was reviewed. That list was put together using a “factor-led” approach. The revisions at this meeting were based on a classical “step-by-step” approach (i.e., transmission/colonization, in vivo growth/evasion of host defenses, tissue damage).

Putative virulence factors:

Transmission/colonization: *cbl* pilus w/22 kDa adhesin

BCESM
Motility?
Antibiotic resistance?
Defensin (cationic peptide) resistance

In vivo growth/immune avoidance: Siderophores?
Antibiotics?
Catalase?
Intracellularly?

Tissue damage: LPS
Supernatant factor?
Hemolytic toxin?
Genomovar-related?

Environmental regulators such as quorum sensing via homoserine lactones and genetic regulation of virulence factors in CF lung infection was discussed as an area in which there are many new developments. Animal models of disease were thought to be critical in further investigations of virulence. Persistence of infection was felt to be essential to development of such models. Currently available models include: (i) intraperitoneal mouse model with splenic persistence; (ii) agar bead model in the mouse and the rat; (iii) cytokine knockout mouse model; (iv) CF mouse model

Molecular techniques which will be of possible utility in the investigation of *B. cepacia* virulence include: (i) transposon mutagenesis; (ii) IVET: in vivo expression technology, using the animal as the selective pressure for gene expression in vivo; (iii) signature-tagged insertion mutants: mutants that do not survive in vivo associated with genes required for infection; (iv) differential display: cDNA in vivo - cDNA in vitro = in vivo expressed genes

Prevention and therapy subgroup

Infection control issues are predominantly an adult problem (pediatric patient population small because of low rates of new acquisition currently and attrition—transfer to adult center, death). The current concept of “cohorting” may be detrimental because of lack of understanding of what makes a given *B. cepacia* strain pathogenic and the potential for acquisition of “more virulent” strains. A policy of “segregation” may be more beneficial. In the hospital this would mean separating patients in private rooms or certainly not placing *B. cepacia*-colonized patients with other CF patients, performing chest physiotherapy in the patient’s room, limiting activities outside the hospital room (including lounges and exercise areas) and educating patients about handwashing and appropriate ways to avoid sharing secretions. In the clinic, options include separate clinics for *B. cepacia*-colonized patients or having patients come at the end of clinic, having patients remain in one room with the caregivers rotating, and using novel strategies such as a pager system to avoid prolonged time in shared waiting areas. For social functions it was felt to be up to the patients and their families to make decisions, but that caregivers should provide enough education and information for patients to make informed choices.

Unanswered questions about infection control include:

- (i) What is the role of intermittently positive or PCR positive patients in transmission of infection?
- (ii) What is the infective dose of *B. cepacia* and does this differ by genomovar?
- (iii) What is the role of genomovar in transmissibility and pathogenicity?

Effective treatment of *B. cepacia* infection remains a difficult problem. It was noted that: (i) we do not have optimal treatment regimen for *B. cepacia*; (ii) we do not know if genomovar is an important variable in

treatment; (iii) we do not know if chronic aerosolized antibiotics are helpful or harmful; (iv) we do not have a validated laboratory method for selection of antibiotic combinations in multiply resistant *B. cepacia*; (v) we do not know the role of inflammation and anti-inflammatory therapy in the management of *B. cepacia* infections.

Questions that need to be further investigated include:

- (i) Are patients with *B. cepacia* exacerbation more likely to benefit from directed vs. empiric therapy?
This could be answered with: (a) a retrospective study, (b) looking at standard practices, (c) a multicenter trial looking at specific outcome measures
- (ii) Is there a role of chronic therapy in the treatment of *B. cepacia* infection?
This could be answered by a randomized, controlled study comparing tobramycin or colistin aerosol with cotrimoxazole oral therapy
- (iii) What is the optimum management of an acute exacerbation caused by *B. cepacia*?
This study could be a prospective trial comparing laboratory-directed vs. empiric combination antibiotic therapy. Patients could be stratified by “susceptible” vs. pan-resistant organism, “cepacia syndrome” or not, and could be evaluated for quantitative microbiology, inflammatory markers, weight changes and improvement in FEV₁.

Genotype/phenotype correlation subgroup

This group initially dealt with the issues of biochemical characterization and the possibility of identification of specific genomovars in the laboratory. Biochemical characterization should be done according to previous recommendations of the group. New caveats include the ability to predict genomovar II (*B. multivorans*) based on sucrose utilization and lysine decarboxylase being negative. The importance of the slow oxidase reaction for identification of *B. cepacia* was reiterated. The question of whether laboratories should be attempting to identify specific genomovars within the *B. cepacia* complex was dealt with: should be performed by reference/referral laboratories, should not be done routinely in clinical laboratories until the clinical significance is clear.

The relationship between genomovar and virulence is not yet defined, but additional clinical data need to be collected by referral laboratories including a short questionnaire with identification of the patient (age, sex, weight, height) and the clinician—this may be melded with CF registry data. In addition, to identify non-pathogenic strains the development of an animal model would be highly valuable. Questions that should be asked include: the stability of virulence factors and what turns on their expression and whether *B. vietnamiensis* and *B. multivorans* are virulent in CF patients (although because they apparently cause severe disease in chronic granulomatous disease, they cannot be considered benign).

It is very clear that a comparison of different identification and typing methods is needed, although perhaps a little premature. A total of approximately 60 strains is suggested including the 30 strains in the “Experimental panel” that is being developed. The current referral labs should be invited to participate as well as other individuals with expertise in these typing methods.

Finally, an appropriate way to distribute the *B. cepacia* experimental panel was discussed. The BCCM/LMG (Belgium Cell Culture Collection) was suggested as a possible source for distribution. Peter Vandamme will contact them about pricing and logistics. This was also suggested as a possible source of revenue for the International Working Group.

Summary of goals for the coming year:

- * Initiation of new investigators to work on *B. cepacia*

- * Distribution of the experimental panel of strains
- * Identification of synergistic/additive antibiotic therapy for *B. cepacia*
- * Exploration of plant products as potentially active antibiotics
- * Performance of a comparative study of identification and typing methods
- * Standardization of genotyping
- * Development of a good animal model for *B. cepacia* infection in CF
- * Encourage deposition of strains in national repositories
- * Final development of *B. cepacia* website
- * Education:
 - identification of organisms, use of selective agar
 - importance of *B. cepacia* in CF
 - segregation of patients as currently optimum method of infection control
 - distribution of information about abstracts, presentations on *B. cepacia*
 - impress upon botanists, agronomists the medical significance of *B. cepacia*

Strategies for future meetings:

- * Tie to another meeting
- * Provide personal monies to attend meeting
- * Publicize meetings
- * Derive monies from marketing of experimental panel
 - * Additional industry support
 - * Less expensive venue, off season
 - * Large city that is easy to get to
 - * Keep annual meetings
 - * Keep attendance high
 - * Organizing committee: Jane Burns, John Govan, John LiPuma, Esh Mahenthiralingam, David Speert