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NTM-TB INSIGHTS

May 2015

National Jewish Health Faculty Science Transforming Life Award Goes To…

Drs. Jerry A. Nick, Milene Saavedra, David P. Nichols, Steve Lommatzsch, and Jennifer Taylor-Cousar
Congratulations!

These physician-scientists from the Adult Cystic Fibrosis Program at National Jewish Health run the largest Cystic Fibrosis (CF) inpatient and outpatient service in the United States. They also have developed an extensive research portfolio, which includes basic science studies, translational research and clinical trials. Working together with a strong sense of collaboration, they have invented novel systems to study the role of the host defense on bacterial biofilm formation, as well as murine models that incorporate these principles to induce chronic infections, a characteristic of CF lung disease.

The team has identified and validated ultrasensitive biomarkers of response to treatment in the setting of CF exacerbation, and markers to predict outcomes in response to treatment of CF and acute lung injury. They have collaborated with others to refine a technique to harvest primary upper airway cells from the nasal passages and propagate these cells indefinitely for the purpose of drug screening and characterization of host cell response. They have established one of few nasal potential difference analysis laboratories in the country, with two certified operators, which allows direct assessment of the cystic fibrosis transmembrane conductance regulator (CFTR) function.

Finally, the group oversees an extremely active clinical research program in which five full-time research coordinators are enrolling patients into 25 active therapeutic or observational trials. In addition to participation on multisite trials, the program has piloted original studies, including a trial of sildenafil for CF lung disease, an innovative "n-of-1" study design of CF gene potentiation that recruited patients nationwide to National Jewish Health, and the first ever pilot studies of a protocolized approach to the diagnosis and treatment of nontuberculous mycobacterial infection in CF patients.

Power and Limitations of Whole Genome Sequencing in Mycobacteria

Mycobacterial whole genome sequencing and analysis efforts have progressed at an accelerated pace, facilitated in part by breakthroughs in next-generation sequencing technologies (1) and improved bioinformatic workflows (2,3). Building on foundational publications of the *Mycobacterium tuberculosis* (4) and *M. leprae* (5) genomes, the research community quickly learned that mycobacterial genomes are diverse in content, have unique gene families (6), and may hold clues that could be leveraged in our fight against mycobacterial pathogens. In addition to the long-recognized mycobacteria of global burden (7,8), genome sequencing efforts have also provided a better understanding of other clinically important mycobacteria and emerging pathogen species of nontuberculous mycobacteria (NTM) (9, 10), including *M. abscessus* (11)*, M. avium* (12)*,* and other species. Just as they have for tuberculosis (13-15), these efforts applied to NTM have helped us better understand pathogen genomic diversity (11), evolution, and mechanisms of drug resistance, and will help us better address epidemiologic questions pertinent to the spread of infection, outbreaks (16,17), and transmission potential (18,19). The question remains, how do we optimally leverage these sequencing capabilities to

not only better understand the pathogens that cause disease, but also to translate this information to clinical needs, including improved diagnostics (7,20), disease surveillance, and drug discovery (21,22)?

Current next-generation sequencing technologies, dominated by the Illumina MiSeq and HiSeq, and the Life Technologies Ion PGM and Proton, are enabling researchers to address epidemiologic and phylogenomic questions at a rapid and robust pace. Complementing traditional methods of species and strain classification using targeted gene sequencing, pulse field gel electrophoresis (PFGE), spoligotyping, mycobacterial interspersed repetitive unit (MIRU) typing, and multilocus sequence typing (MLST) (20), whole genome sequence information allows us to differentiate and identify differences at the genome level by comparing single nucleotide polymorphisms throughout the genome or within the core genome, even among closely related strains or species. These methods can help us tease apart the genetic relatedness and phylogeny of bacterial pathogens, and also help us to develop hypotheses pertinent to genotype-phenotype relationships, including genetic contributors to drug resistance. Using these methods, we can rapidly identify single nucleotide polymorphisms and larger genomic differences among strains, for use in genomic comparisons, phylogenomic analyses, and genotype-phenotype analyses. Genome comparisons can also be used to identify mechanisms of action of new drug candidates (22). In combination with phenotypic susceptibility testing, these methods may pave the way to a new generation of molecular mycobacterial diagnostics.

As with any technology, not all that glitters is gold. Although next-generation sequencing has provided a significant research tool for whole genome bacterial sequencing, there are still limitations that need to be overcome and addressed. First, the most dominant next-generation sequencing platforms still rely on relatively short sequencing reads, between 50 base pair (bp) and 400 bp reads, have platform nuances including guanine-cytosine (GC) content sequencing bias, and homopolymer and indel calls are still not as robust as one might desire on certain platforms (23). These factors are important to consider with mycobacterial *de novo* genome sequencing and resequencing efforts, since mycobacterial genomes are typically GC rich, have large gene families with multiple paralogs, and sequence library construction is sometimes challenged by mycobacterial nucleases or inefficient lysis of the recalcitrant mycobacterial cell wall. Some of these challenges may be addressed by longer-read, single molecule and nanopore sequencing technologies, including those emerging from Pacific Biosciences and Oxford Nanopore.

Data analysis strategies are also important to consider, since genome quality is impacted by a combination of sequencing coverage and quality, assembly and mapping methods, and other bioinformatic strategies. Many of these limitations can be overcome by carefully planned experiments and strategies, including hybrid assemblies utilizing more than one sequencing platform, adequate coverage depth thresholds for polymorphism calls, sequence quality filtering and trimming, and other methods, providing us with high quality sequences for *de novo* genome assembly, genome resequencing, and subsequent analyses.

Together these methods can tell us much about pathogen evolution, phylogeny, drug resistance mechanisms, and genome plasticity, but we do need to work to translate this information for improved diagnostics, disease surveillance, and to facilitate drug discovery. As the cost of next generation sequencing continues to decrease, we are likely to see increasing clinical and research applications. It is an exciting time for mycobacterial genome sequencing, proving renewed hope in our quest to better combat and limit deadly mycobacterial infections.

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50 Years Ago – Aspects of Bacterial Resistance in Tuberculosis

The J. Burns Amberson Lecture presented by George Canetti at the American Thoracic Society Conference in 1965 (*Am Rev Respir Dis*. 1965. 92:687-703)

I. Resistance and Bacillary Populations

The reason I have chosen bacterial resistance in tuberculosis as the subject for this lecture is not merely because I have done some work in the field, but because it is my strong belief that a revival of active interest in resistance is needed. The neglect into which this most promising field of research has fallen is amazing. Bacterial resistance is as old as antituberculosis chemotherapy.

The main biologic facts, such as the existence of resistant mutants in wild strains, their probable selection by treatment under certain conditions, the link between resistance and severe tuberculosis, the prevention *in vitro* of resistance by the use of two drugs, were all shown within the first five years. Once several drugs had become available, controlled clinical trials assessed, *in vivo*, the value of combined regimens in increasing the efficiency of chemotherapy and preventing the emergence of resistance. Important basic facts, such as the diminishing virulence of isoniazid-resistant strains, the link between enzymatic deficiencies and these virulence changes, the existence of drug-susceptible 'persisters' after long-term chemotherapy in experimental murine tuberculosis, were added. This all occurred within the next five years. And then the impetus faded away.

Resistance is fundamentally a phenomenon linked to large initial bacillary population. In human tuberculosis the greatest populations are those prevailing in cavities (1): the far greater frequency of emergence of resistance during treatment of cavitary tuberculosis, compared with noncavitary tuberculosis, was shown as early as 1949 (2,3), and is now common experience. Data derived from patients submitted to resection without prior chemotherapy – through diagnostic error, for instance – have permitted estimations of the total bacillary population initially present in different types of lung lesions. The populations found in cavities were of the order of 10^7 to 10^9 bacilli, whereas those found in hard caseous foci, the most common type of lesion, did not exceed 10^2 to 10^4 bacilli (4).

According to the generally accepted theory, resistance appearing during drug treatment is due to the selection and multiplication of the resistant mutants pre-existing in the bacillary population of the host. Inasmuch as the simultaneous destruction of the susceptible part of the population involves an incomparably greater number of bacilli – provided the

infecting strain is normally susceptible to the drug employed – the overall consequence is a sharp drop in the total population during the initial period of treatment; the rise due to unhampered multiplication of the resistant mutants reveals itself later. This 'fall and rise' phenomenon, as demonstrated in the patients' sputum, was first described very early by Pyle (5) and by Mitchison and Crofton (6,7). Its practical importance is obvious, as it sometimes permits detection of rising resistance by a procedure as simple as microscopy of the sputum. Of course, there must be certainty of the actual taking of the drug by the patient.

Although bacillary multiplication is a fundamental process in the emergence of resistance, cases in which the residual foci harbor extremely small, resistant populations after prolonged chemotherapy undoubtedly exist. Among a total of 415 unsterilized cavitary resections (4) in patients who had undergone chemotherapy for more than a year, the residual cavitary focus, of the cleansed or inspissated type, showed such populations in 60 cases (14%). These populations, usually 1 to 10 colonies for the huge inoculum chosen, are so small, compared with the initial populations of cavities, that the bacilli involved indeed deserve the name 'persisters'. Most often these bacilli do not appear in the sputum. They may be locked in by closure of the draining bronchus; cleansed cavities show a closed bronchus in one-third of the cases and inspissated cavities in two-thirds. But, even when the bronchus is open, the extreme scarcity of the populations and the usual absence of bronchial secretion in such cases, in which all inflammatory processes have long since subsided, provide a sufficient explanation for the clinical undetectability of these residual persisters.

The ultimate fate of the resistant persisters is variable. They may start multiplying one day and lead to clinical relapse, just as drug-susceptible persisters do. In an appreciable number of the bacteriologic relapses occurring in patients whose sputum has become negative without showing at any time resistant bacilli, the strain found at the time of relapse is resistant (8,9). However, the temporary presence of a few resistant persisters in the sputum, as described above, does not itself mean relapse; and whether true relapse, that is the lasting presence of bacilli in the sputum, occurs more often in such cases is unknown. In all likelihood, spontaneous death of the persisters is the outcome in many cases.

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Meetings

Carolyn and Matthew Bucksbaum NTM Lecture Series for Physicians, Patients, and Families, Friday May 15, 2015; An educational collaboration with National Jewish Health and NTMir. History Colorado Center, 1200 Broadway, Denver, CO 80203. Free registration. Click here for more information and registration.

American Thoracic Society Public Advisory Roundtable (PAR) ninth annual patient/family forum; Saturday May 16, 2015 from 10:00 a.m. to 2:00 p.m. Sheraton Denver Downtown Hotel. Free registration.

American Thoracic Society Microbiology, Tuberculosis and Pulmonary Infections (MTPI) Membership Meeting on Monday, May 18th, 2015: Embassy Suites Silverton Ballroom 1&2 (Second Floor) from 5:00-7:00 pm.

9th National Conference on Laboratory Aspects of Tuberculosis, Association of Public Health Laboratories (APHL), June 8-9, 2015 at the Grand Hyatt Hotel Buckhead, Atlanta, GA. Click here for more information and registration.

National Tuberculosis Conference, National TB Controllers Association (NTCA), June 9-11, 2015 at the Grand Hyatt Hotel Buckhead, Atlanta, GA. Click here for more information and registration.

The 52nd Semi-Annual Denver TB Course, October 14-17, 2015; Molly Blank Conference Center at National Jewish Health Main Campus. Click here for more information and registration.

20th Annual Conference of the International Union Against Tuberculosis and Lung Disease – North American Region, February 25-27, 2016. Sheraton Denver Downtown Hotel.