Molecular Epidemiology of *Pneumocystis carinii* Pneumonia

Pneumocystis carinii pneumonia (PCP) was first recognized as a distinct clinical entity in orphanages in Europe during World War II (1). Today it is the most frequent serious opportunistic infection in AIDS patients. Despite advances in research, numerous questions remain regarding the basic biology and epidemiology of *P. carinii*.

Transmission and Patient Care

Although reactivation of latent infections has long been considered the primary explanation for PCP in immunosuppressed patients, over the years a steady flow of reports has described clusters of PCP cases (2). In addition, recent studies have suggested that the duration of latency is very limited, i.e., usually less than 1 year (3,4). Still other studies have demonstrated genetic variation in PCR-amplified *P. carinii* DNA from the lungs of patients during subsequent PCP episodes (5). Together, these observations provide strong circumstantial evidence of person-to-person transmission of *P. carinii*. Consequently, establishing the role that person-to-person transmission plays in the epidemiology of PCP is urgent.

Another important area of PCP epidemiology is determining the predisposing factors for disease. The most frequently discussed predictor of disease is CD4+ cell count, specifically as it relates to care and management of AIDS patients (6); however, it has long been known that malnutrition can be an important contributor (7). The degree to which other factors such as viral infections or pneumonitis of other causes, may come into play, is yet to be shown.

Much can also be learned regarding the epidemiology of PCP in HIV-infected infants. Recent studies report that primary infections in these infants often develop when the child is 3 to 6 months old (8,9). The source of these patients' *P. carinii* infections (i.e., the hospital setting, their mothers, other children, or an environmental source) is not known.

Clinicians working with AIDS patients need a sensitive, reliable, and noninvasive tool for early detection and diagnosis of PCP infections (10,11). Besides the standard procedures of bronchoalveolar lavage (BAL) and induced sputum (IS) sampling, recent studies indicate that it is possible to amplify *P. carinii* DNA sequences by polymerase chain reaction (PCR) directly from blood or serum samples and from nasopharyngeal aspirates of PCP patients (11,12). Further studies are needed to confirm that a PCR-based diagnostic tool superior to microscopy can be adapted for use in clinical settings. A serologic tool that will distinguish recent PCP infections from those past is also needed.

Prophylaxis failures have been reported for both trimethoprim-sulfamethoxazole (TMP-SMX) and pentamidine (13-16). Studies evaluating these cases, however, are frequently complicated by the difficulties in assessing and confirming patient compliance with the prophylaxis regimen. The only factor that has a significant correlation with failure in most cases, however, is the patient's CD4+ T lymphocyte count (14). Although this correlation would be expected because of the general increased risk for PCP associated with CD4+ cell depletion (6) and the increase in prophylaxis complications in HIV-infected patients (17), these drugs may not eliminate all organisms, and some degree of patient immunity may be required to clear or control the infection. What role, if any, specific antimicrobial resistance mechanisms play in the reported treatment failures has not been shown; however, the emergence of resistance is always a threat. Likewise, long-term TMP-SMX prophylaxis increases the possibility for the selection of antimicrobial resistance in bacterial pathogens, some of which are important potential causes of pneumonia in HIV-infected patients (18). Identifying potential antimicrobial resistance mechanisms in *P. carinii* is difficult because of the lack of an established culture system for human *P. carinii* that would allow traditional antimicrobial sensitivity testing.

At least three separate lines of data suggest that *P. carinii* is a commonly encountered organism: the high seroprevalence rates reported in normal populations (19), the rapid rate at which infants acquire primary infections (8) and AIDS patients become reinfected after successful treatment (20), and the amplification of *P. carinii*-specific DNA from ambient air sampled from the environment (e.g., an apple orchard) (21) and from rooms of animals and patients with PCP (22). Airborne transmission has been demonstrated for PCP in rats (23-26) and is by far the most likely mechanism proposed for natural exposure to *P. carinii* in humans (2,22). Given the similarities

between *P. carinii* and various fungal agents and the enigma surrounding the issue of environmental sources for *P. carinii*, it has been suggested that *P. carinii* may in fact be a dimorphic fungus, ubiquitous in the environment and disseminated by airborne spores (27). Identifying the specific environmental source or sources of *P. carinii* is critical to understanding the epidemiology of PCP and establishing guidelines for preventing its transmission.

It is generally accepted that *P. carinii* strains from rats do not infect humans and that human strains do not infect rats; however, we do not know the host boundaries for a given *P. carinii* strain or if all isolates from a given host display the same degree of host restrictions (28,29). In fact, a careful evaluation of the available data concerning *P. carinii* of numerous hosts suggests that *P. carinii* may represent a collection of diverse fungal species (30). Like drug resistance research, studies aimed at strain/species characterization are generally hindered by the difficulties in culturing human *P. carinii* and the lack of refined molecular biological methods that allow strain identification and characterization.

Molecular Biologic Techniques and Specific Epidemiologic Issues

One of the essential reasons for cultivating any particular pathogen is for strain identification and characterization that would elucidate such specific phenotypic characteristics as virulence factors, antimicrobial sensitivity levels, and factors associated with transmissibility. The isolation and cultivation of individual strains, and ultimately of clones, would provide a homogeneous population of organisms from which the desired information can be obtained and a pure source of genetic material for constructing DNA libraries and identifying relevant genes.

In the absence of cultivation, investigators have been able to begin addressing some of the basic epidemiologically important issues by applying PCR-based technology. In these studies, the DNA sequence of specific genetic loci from *P. carinii* is usually amplified from BAL, IS, or serum samples from PCP patients, using highly specific oligonucleotide primers. Inherent problems exist in this approach (which are discussed below); however, the approach has allowed the identification of genotypic differences in *P. carinii* populations sampled from the lungs of different patients and even from the lungs of the same patient during different PCP episodes. Great potential exists in applying this technology to develop molecular profiles of *P. carinii* isolates that could ultimately allow the particular genotypes to be linked to specific epidemiologically relevant phenotypes.

Molecular Typing

Five to ten different genetic loci have been identified as potentially informative for molecular characterization and typing (30-33). Concerning the typing that has actually been performed on human samples, the primary loci evaluated include: 1) a 346-bp region of the mitochondrial large subunit rRNA gene (*mt lsrRNA*) (10) and 2) a 550-bp fragment containing the nuclear ribosomal internal transcribed spacer regions 1 and 2 (*ITS1* and *ITS2*) (34). When these loci are considered collectively, nucleotide variation can be detected at approximately 37 different positions. Work is in progress in several laboratories, both to type patient isolates according to the available loci and to identify additional genetic loci to more thoroughly define a given genotype.

The primary obstacles to the development of a molecular typing scheme based on PCR-amplified DNA sequence data obtained from PCP patients include the following: 1) multiple strains may infect a single patient at a given time; 2) a diploid organism of a single strain may be heterozygous with respect to a particular polymorphic locus; 3) presumed single genes could have multiple copies in a single genome, which could give the appearance of genetic polymorphism; 4) amplified DNA sequence data might be confounded because of other fungal agents such as *Cryptococcus* or *Candida*; and 5) inferences that can be drawn from restricted sequence data (i.e., gene typing versus strain typing) are limited. Although these problems are not insurmountable, they must be considered when evaluating data obtained by this approach. We propose the following recommendations.

Recommendations

Molecular Epidemiology

1. Recent advances in molecular-based typing should be combined with epidemiologic studies to investigate the transmission of *P. carinii* and new strategies for control.

2. Additional genomic regions must be identified for use in typing, along with the genetic loci that are available. These new loci must be shown to represent single-copy genes. Also, new molecular approaches should be developed that will increase the current capacity to resolve genotypic variation among *P. carinii* strains.

3. Genetic variation should be investigated among *P. carinii* strains that could be linked to variations in factors such as strain virulence, drug resistance, or transmissibility.

4. The critical issue regarding person-to-person transmission is not so much whether it occurs, as whether it contributes to infection significantly more than airborne sources in the environment. Thus, it must be determined whether there is any benefit to establishing complex protocols that ensure that patients are carefully protected from each other if they can become infected from other sources in the environment. Consequently, the importance of person-to-person transmission in the epidemiology of PCP should be defined.

5. The role of latent *P. carinii* infection as a source of PCP in immunocompromised persons should be clarified.

Diagnosis, Treatment, and Prevention

1. New tools for noninvasive early diagnosis of PCP, including culture systems, molecular approaches, and serologic tests that can distinguish recent and past PCP infections are needed.

2. In the United States, clinician compliance with recently published U.S. Public Health Service/Infectious Diseases Society of America guidelines on the treatment and prophylaxis of PCP should be evaluated.

3. Studies should be initiated to develop additional drugs for PCP treatment and prophylaxis.

4. New approaches for improving patient compliance with prescribed PCP prophylaxis must be devised and evaluated.

5. Methods for detecting the possible emergence of drug resistance to *P. carinii* should be standardized.

6. Standard decontamination procedures for respiratory therapy equipment and pulmonary diagnostic instruments should be evaluated to confirm that they effectively eliminate all viable *P. carinii*.

Environmental Reservoirs and General Biology

1. Environmental sources and the coinciding infective stage(s) of *P. carinii* should be detected and evaluated.

2. The host range of *P. carinii* from various sources (i.e., to what degree are humans susceptible to *P. carinii* from nonhuman sources?) should be determined.

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Needed: Comprehensive Response to the Spread of Infectious Diseases

In his article "Globalization, International Law, and Emerging Infectious Diseases," Fidler recognizes that biological agents travel by themselves or with their hosts without any recognition of, or regard for, political borders. He notes that with the continued expansion of economic commerce across continents and more rapid transport and travel, persons infected with infectious diseases of very short incubation periods can act as vectors across several nations before they even become symptomatic. The protective effect of clipper ship travel is long gone.

Fidler examines the need for international treaties, agreements, and policies to manage the spread of new or reemerging infections diseases. His concern is that the current international climate requires more enforceable treaties with adequate resources to identify, track, interfere with, and contain the spread of infectious diseases perceived as an international or global threat.

International cooperation within the existing legislative mechanisms has, on occasion, been very successful. International eradication of smallpox was successful because a specific, cost-effective, efficient vaccine was developed; the disease attacked persons regardless of their economic, political, racial, religious, or social affiliations; the amount of funding was adequate; and all nations recognized the benefits of the eradication program. A similar effort currently in progress to eradicate poliomyelitis will also be successful because of international cooperation.

In contrast, international control of other infectious diseases, such as malaria and tuberculosis, has been attempted for decades with considerably less success. Notwithstanding the lack of efficacious vaccines, the reality is that only very limited resources are being committed to prevent and treat all infectious diseases. Outbreaks of Ebola virus infection and plague are routinely reported in the local, national, and international press. However, the continued increased incidence and prevalence of tuberculosis, AIDS, and other sexually transmitted diseases are accepted by many as problems of the poor, the immoral, and the expendable portion of society. Local, national, and international awareness and continued interest are significant problems.

International cooperation must extend beyond merely restricting the natural spread of specific diseases. One also has to recognize the need for effective international treaties to prevent the use of biological agents in either tactical or strategic circumstances. Fear of combatants using biological agents on military and civilian targets intensified during and since the Gulf War. The possibility of biological terrorism is no longer limited to the imagination of fiction writers. Fidler does not stress the issue of nonnatural outbreaks of diseases; a global need for an improved non-ad hoc response to emerging infectious disease agents should be completely considered by civilian and military planners. The threat of infectious diseases as weapons provides an additional incentive for cooperation among governments.

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