

MEETING REVIEWS

Why the International Workshops on Opportunistic Protists?[∇]

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After human immunodeficiency virus (HIV)/AIDS was recognized in the 1980s as a pandemic impacting the United States and Western Europe, it was discovered that in many cases, several microbes rather than HIV itself were the direct cause of mortality. Given the importance of these microbes in AIDS, immunodeficiency-associated diseases (IDADs) and opportunistic infections (OIs) caused by several eukaryotic protists have received the attention of biologists and clinicians. These organisms, however, have been particularly difficult to work on or manipulate experimentally. They represent diverse taxa such as microfungi (*Pneumocystis*, microsporidians, *Candida*, *Histoplasma*), apicomplexans (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*), and other unicellular eukaryotes (free-living amebae, *Blastocystis*) but present similar challenges to investigators. The difficulties faced with most of these organisms are primarily based on the lack of in vitro axenic mass culture methods that would allow rapid organism proliferation and indefinite subcultivation. Various immunodeficient animal models and cocultivation methods with animal cell lines have been developed, but these alternatives have not eliminated the challenges of low organism yields, serial subcultivation, and facile genetic manipulations. Hence, despite “brute force,” expensive, labor-intensive, and creative research approaches, there remain many unanswered questions.

The infectious forms of some of these organisms are not yet fully understood (e.g., *Pneumocystis*) or there remains uncertainty about the species or genotypes that are able to infect humans (e.g., *Cryptosporidium*, *Enterocytozoon*). While these OIs are pathogenic in severely immunocompromised hosts, they are also able to infect those who are immunocompetent. Only limited data are available on the impact of these infections in immunocompetent hosts and the potential roles of these hosts in spreading these pathogens in host populations (e.g., *Pneumocystis*, microsporidia). There are still limited reproducible animal models, which hinders the understanding of the pathophysiological mechanisms in these infections. The lack of effective drugs against these eukaryotic OIs remains a major concern, and pharmaceutical drug development is still limited. For example, there are only a few effective drugs against *Pneumocystis* pneumonia (PcP) and some have serious side effects. Also, no safe drugs that efficiently clear *Cryptosporidium*,

Enterocytozoon bieneusi, *Toxoplasma*, or amebic encephalitis infections are available.

International Workshops on Opportunistic Protists (IWOP). Initially in 1988, *Pneumocystis* was the only pathogen discussed at IWOP, as it was the greatest immediate cause of death among AIDS patients in the Western world, and it remains a significant cause of morbidity and death among these individuals. It soon became evident to the IWOP organizers that investigators working on other AIDS-associated eukaryotic OIs shared similar challenges. By IWOP-10, investigators working on *Cryptosporidium*, microsporidia, free-living amebae, and *Toxoplasma* had joined this conference. Upon its inception, the workshops have been open to anyone who wishes an audience for presenting data on these organisms and the diseases they cause, as well as to observers seeking information on the current status of research on these pathogens. The meetings have proved conducive for the exchange of new information, including discussion of the difficulties investigators have encountered. This forum has been used for reaching agreement on the standardization of research protocols and for initiating and strengthening collaborations. IWOP also serves as a forum where consensus on issues such as organism nomenclature can be discussed and resolved. For example, consensus for adopting an interim trinomial nomenclature for *Pneumocystis* was achieved at IWOP-3 (2) and consensus on the *Enterocytozoon bieneusi* internal transcribed spacer (ITS) genotype designation was reached by microsporidia researchers at IWOP-10. Endorsements for elucidating the *Pneumocystis carinii* genome by an international group of investigators and related collaborations were started at IWOP-5 in 1997, which helped launch the *Pneumocystis* Genome Project. Projects for sequencing microsporidia (6), *Cryptosporidium* (1, 19), and *Toxoplasma* (13) have also been worked on by several participants of IWOP, and progress on those projects was presented to this audience. Information from such genome projects has provided a powerful means by which the basic biology of these pathogens can be explored in lieu of direct experiments on the organisms themselves.

New insights, changing demographics. There is a growing appreciation that infectious diseases need to be understood in the context of the environment and ecology, evolution and coevolution, climate change, and the speed at which people and goods are transported globally. It is known that the microbiota of humans and domestic animals are diverse and immense, indicating the potential of some organisms to become pathogenic if the environment in or on the host becomes altered. A better understanding of the natural history of OIs that emerged with AIDS provides insights into the nature of

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[∇] Published ahead of print on 23 January 2009.

the enormous numbers of microbes passing through or inhabiting our bodies. Some are observed to be beneficial commensals, others are potential pathogens that can cause asymptomatic infections in resistant hosts, and yet others are efficiently cleared by the host's natural defense system. Questions still exist about whether these symbiotic relationships represent coevolved host-parasite interactions resulting in asymptomatic transient infections or whether infections may possibly reactivate under appropriate conditions (e.g., aging). Information is lacking on the natural ecological niches in the host where heterogeneous collections of pathogenic or commensal microbial populations coexist, which can be evaluated by metagenomic analyses on assemblages from defined environments (10). A better understanding about how the natural balance is established in healthy people may contribute to our understanding of the basic pathophysiology of these infections, and hence how to treat people with defective immune systems who exhibit disease.

Although *Pneumocystis* was recognized as an agent of severe pneumonitis in premature or weakened young children well before the AIDS pandemic, this and several microsporidia species are currently described as having emerged with AIDS, which suggests that these pathogens are probably more prevalent in humans than is currently appreciated. If it were not for AIDS, we would know far less about these infections than we now know, and it appears that several of these pathogens are fairly common. Tools, such as sensitive serological assays for detecting *Pneumocystis* antibodies, have been developed (9) and seroepidemiological data indicate that nearly all people in both developed and underdeveloped countries have had colonizations or frank infections. There is also evidence for high prevalence rates of microsporidiosis among healthy people with or without diarrhea, which is particularly true of those living in underdeveloped countries with sanitation problems (16). Even in developed countries, outbreaks and spread of enteric diseases such as those caused by *Cryptosporidium hominis*, *Enterocytozoon bieneusi*, *Encephalitozoon* species (e.g., *E. intestinalis* and *E. cuniculi*), and *Giardia lamblia* could become commonplace if rules and guidelines for wastewater, drinking water, and recreational water are not strictly implemented and regulated. *Naegleria* and other infectious free-living amoebae are commonly found in drinking and recreational waters, but why more people do not get infected remains a mystery. We lack rapid diagnosis and treatment for free-living amoebal infections; the current diagnosis of amoebic encephalitis is primarily done upon autopsy. Unknowns about future scenarios include, for example, whether the prevalence of amoebic encephalitis would increase with global warming. In addition, these infections are of particular concern among immunodeficient populations, since there are still no good drugs for safely clearing these enteric and central nervous system OIs.

In recent years, changes have been observed in the pattern of AIDS-related diseases in developed countries. For example, the introduction of PcP prophylaxis and highly active antiretroviral therapy has resulted in a decline of AIDS-related PcP (15). However, PcP has increasing importance in HIV-negative patients treated by immunosuppressive treatment. The incidence of PcP is increasing, and higher mortality rates of 40% to 50% are being observed in these patients (3, 14, 21). The course of disease and outcome from PcP are worse in these

immunosuppressed patients, and an HIV-negative status appears to be an independent predictor of mortality in severe PcP cases (14). IDAD agents are also finding new habitable niches, such as in patients undergoing tumor necrosis factor alpha therapies (e.g., infliximab), thus broadening the range of susceptible patients to now include those with rheumatoid arthritis and Crohn's disease (8, 12, 18, 22).

Lack of good drugs against opportunistic protist infections. Despite our ability to model three-dimensional structures of target molecules for designing novel drugs; the development of efficient, high-throughput screening assays; the ability to mine data from genomic, proteomic, and metabolomic analyses; and the development of other modern technologies, there has not been a single novel class of antibiotics marketed since 1970 (17). It has been suggested that despite the recent sequencing of hundreds of bacterial genomes, this approach for discovering good antibacterial drug targets has not been fruitful. Does this also predict the utility of the genomic approaches for the development of chemotherapy against protistan infections? Although we know much more about eukaryotic AIDS-associated OIs, we are nowhere close to really understanding the basic biology of these microbes nor have we significantly succeeded in enhancing the rapid diagnosis and cure for these diseases in either wealthy or impoverished countries.

Funding support of IDAD research. There appears to be a growing consensus that fundamental research on the basic biology of pathogens is what is urgently needed. However, funding for such "descriptive," non-hypothesis-driven research has not surged. Research on these important pathogenic infections is given special consideration by some funding agencies that recognize the difficulties investigators face by working on these intractable but important organisms. That is to say, the state of the art in the research on these organisms cannot be expected to be at a level of sophistication comparable to those of the cultivable causative agents of malaria, leishmaniasis, sleeping sickness, and Chagas' disease. The problem in research funding for these IDAD pathogens is similar to that generally faced by parasitological research as a whole. With the recent exception of malaria (e.g., Rollback Malaria), parasite and fungal infections have long been considered by the biomedical field as "third world," "neglected," "tropical" (11), or "miscellaneous" diseases, and drugs against such diseases are often considered to be "orphan" drugs. Funding for these eukaryotic IDADs is, in fact, even more dismal than the support of research on the traditional neglected tropical diseases. Less is known about the basic biology and natural history of free-living or commensal organisms, despite knowing full well that all organisms are in fact opportunistic and could find a suitable niche within or on humans, if the situation permits. It should be noted that the ubiquitous pathogenic protists of interest to IWOP participants (fungi and protozoa) occur worldwide and that these are quite cosmopolitan in distribution (5). Human infections caused by several of these pathogens are being increasingly detected in otherwise healthy people.

Since some transient infections in healthy people can become fulminant and life-threatening when the host's immune system becomes compromised, continued studies are needed to more fully understand their biochemistry, cell biology, molecular biology, immunopathology, epidemiology, ecology, and mechanisms of transmission, so that intervention strategies can

be employed, including the development of drugs for effective treatment and approaches that reduce the emergence of drug resistance. Compared to diseases caused by viruses and bacteria, diseases caused by eukaryotes are often more difficult to cure because the organisms are more closely related to their human and animal hosts. Therefore, many available chemotherapeutic drugs that are lethal for the pathogen exhibit toxicity in the host. It seems almost impossible to develop effective vaccines against these relatively large and complex organisms, in contrast to the relatively rapid and easy production of vaccines against viruses and bacteria. Although it is generally known that infections tax the host's defense systems, little is known about the pathogenesis and treatment of multiple infections (polyparasitism), which is common in the tropics and the developing world (4) and might be more prevalent than suspected in developed countries.

OIs will emerge and reemerge in the future. There is concern that as the AIDS pandemic plateaus, OIs will become increasingly neglected by the scientific community before effective therapies have been developed. On the other hand, emergent and reemergent infections have increased within the past two decades or threaten to increase in the near future (7). It is believed that immunosuppression (due to aging or chemotherapy, for example), AIDS, and hospitalization are much more important factors favoring the emergence or reemergence of infectious agents than are genetic mutations, globalization, or climate change (20).

IWOP plays a uniquely important role within health care systems as the population of immunodeficient and aging people increase, not only through the spread of HIV globally but also because advances and treatments of cancer and solid organ transplantation involving immunosuppressive therapy become more commonplace. The population of elderly people is increasing, and immune status in these individuals often declines. This may lead to increased IDADs in these individuals, thereby causing a public health concern.

The cosmopolitan nature of the three dozen fungal and protozoan infectious agents that are causing emerging and reemerging diseases is important information for the Western world (5). Data indicate that developing and developed countries have approximately similar numbers of emerging and reemerging pathogenic protists; the highest number of species is actually found in North America (20). As many of these organisms cause IDADs, the pathogens of interest to IWOP are becoming less and less restricted to tropical regions of the world, and it would be prudent for the Western world to not neglect them. An encouraging sign that research on these "difficult to work with" pathogens will not become even more neglected is the high proportions of enthusiastic students, postdoctorals, and young investigators with diverse backgrounds who are not discouraged by investigative challenges and continue to participate in IWOP.

ACKNOWLEDGMENTS

Preparation of this article was supported in part by grant RO1 AI064084 to E.S.K. and was made possible by grant R13 AI078718

from the National Institute of Allergy and Infectious Diseases Division of AIDS.

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