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The 12th International Workshops on Opportunistic Protists (IWOP-12)

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Abstract

The 12th International Workshops on Opportunistic Protists (IWOP-12) was held in August 2012 in Tarrytown, New York. The objectives of the IWOP meetings are to: (1) serve as a forum for exchange of new information among active researchers concerning the basic biology, molecular genetics, immunology, biochemistry, pathogenesis, drug development, therapy, and epidemiology of these immunodeficiency-associated pathogenic eukaryotic microorganisms that are seen in patients with AIDS and (2) foster the entry of new and young investigators into these underserved research areas. The IWOP meeting focuses on opportunistic protists, e.g. the free-living amoebae, *Pneumocystis*, *Cryptosporidium*, *Toxoplasma*, the Microsporidia, and kinetoplastid flagellates. This conference represents the major conference that brings together research groups working on these opportunistic pathogens. Slow but steady progress is being achieved on understanding the biology of these pathogenic organisms, their involvement in disease causation in both immune-deficient and immune-competent hosts, and is providing critical insights into these emerging and

reemerging pathogens. This IWOP meeting demonstrated the importance of newly developed genomic level information for many of these pathogens and how analysis of such large data sets is providing key insights into the basic biology of these organisms. A great concern is the loss of scientific expertise and diversity in the research community due to the ongoing decline in research funding. This loss of researchers is due to the small size of many of these research communities and a lack of appreciation by the larger scientific community concerning the state of art and challenges faced by researchers working on these organisms.

Keywords

Acanthamoeba; Blastocystis; Cryptosporidium; Giardia; *Microsporidia*; Pneumocystis; Toxoplasma

This article presents a summary of research talks and posters presented at the 12th International Workshops on Opportunistic Protists (IWOP-12) that was held on 5–9 August at the Tarrytown House Estate and Conference Center, Tarrytown, New York. The data presented by the participants covered significant advances in, and major challenges of, research efforts in understanding the biology of immunodeficiency-associated opportunistic protists and their interactions with animal hosts. Abstracts of all presentations are available at <http://www.einstein.yu.edu/labs/louis-weiss/?id=34265> and citations in the text indicate talks [T#] or poster sessions [PA# or PB#] in this abstract book.

PLENARY SESSION AND ROUND TABLE DISCUSSIONS

Dr. Peter Walzer received a lifetime achievement award from the International Workshops on Opportunistic Protists for his pioneering work on pneumocystosis. He presented a provocative plenary lecture on “Pneumocystosis: Perspectives on the Past and the Future” [T1]. In addition to a brief review of his work on pneumocystosis and on the development of this field of research, he discussed the understudied role of environmental factors in the transmission of this infection and disease penetrance. *Pneumocystis* is an opportunistic fungal pathogen found in the lungs of humans and other animals in nature. Research on *Pneumocystis* has been hampered by the lack of a reliable in vitro culture system. It is an important cause of pneumonia in immunocompromised hosts. *Pneumocystis* respiratory tract colonization has been associated with a decline in lung function. One of the least studied areas of *Pneumocystis* research is its ecology. *Pneumocystis* can be transmitted by close contact and the airborne route, and the cyst (now referred to as ascus) is the likely transmissible form. It is also possible that an additional, undetected stage may exist in the air. The effects of environmental factors on cyst viability are poorly understood. Outbreaks of pneumocystosis are occurring more frequently in renal transplant recipients than in the past, and geographic clusters of pneumocystosis have occurred in HIV+ patients. Studies of HIV+ patient populations have identified temperature and outdoor activities (hiking, gardening) as independent risk factors for pneumocystosis. More basic and clinical research is needed to better understand the interaction of this pathogen and the environment. The use of tools that are already available or adapted from studies of other organisms is a first step to achieving this goal.

Brian Brunk presented data and provided ongoing individualized teaching during the entire 12th International Workshops on Opportunistic Protists (IWOP-12) meeting on the use of the Eukaryotic Pathogen Genome Database (<http://www.EuPathDB.org>), an NIH/NIAID-funded Bioinformatics Resource Center, which provides an online portal to the complete genome sequence, annotations, and integrated functional genomics data from multiple eukaryotic pathogens [T2]. These include *Entamoeba* spp. (AmoebaDB. org),

Cryptosporidium spp. (CryptoDB.org), Microsporidia (MicrosporidiaDB.org), *Babesia* spp. and *Theileria* spp. (PiroplasmaDB.org), *Plasmodium* spp. (PlasmoDB.org), *Toxoplasma gondii*, *Neospora caninum* and *Eimeria tenella* (ToxoDB.org), *Giardia* (GiardiaDB.org), *Trichomonas vaginalis* (TrichDB.org), and kinetoplastids (TriTrypDB.org). The EuPathDB component databases enable scientists to ask questions about complex genomic-scale data using an intuitive graphical interface.

Dr. Robert E. Molestina presented data on the holdings and resources available at the American Type Culture Collection (ATCC) that can support research on opportunistic protists [T3]. Deposition of any organisms used for a genome project was discussed. Strains deposited in culture collections are considered “biological standards” as they are critical components of comparative studies. The Protistology Collection at ATCC houses the largest and most diverse repository of parasitic protozoa in the world. The primary role of the collection over the years has been the acquisition, authentication, preservation, and distribution of reference parasite strains to the wider scientific community. Characterization of deposited cultures includes a variety of tests such as viability, purity, phenotypic properties, and genotypic analysis. Establishing seed and distribution stocks for every strain is a common practice to make certain that cultures distributed to researchers are closely similar to the original material provided by the depositor. Over the last decade, resources within the collection have expanded to include a variety of biomaterials such as genomic DNAs, polyclonal antisera, monoclonal antibodies, and expression clones.

Roundtable discussion: the AIDS-Associated Opportunistic Infections and Cancer Study Section

Spearheaded by Dr. Kami Kim, recent chairman of AIDS-Associated Opportunistic Infections and Cancer (AOIC) Study Section, this was a discussion on the funding issues at NIH, the mechanism for review and referral of grants to AOIC, issues related to concerns about future funding for opportunistic research, and the loss of scientific expertise due to laboratory closings as a consequence of the funding environment. As described on the Center for Scientific review website <http://public.csr.nih.gov/StudySections/IntegratedReviewGroups/AARRIRG/AOIC/Pages/default.aspx>) “The AIDS-associated Opportunistic Infections and Cancer [AOIC] Study Section reviews applications on opportunistic infections and cancers associated with HIV and AIDS. The science encompasses pathogenesis, immune responses, animal models, and molecular characterization of AIDS-associated opportunistic infections and cancers. Proposals should address opportunistic infections in the context of HIV infection/AIDS” and the grants relevant to AOIC include those that investigate: “Molecular, cellular, and tissue-based studies of pathogenesis of AIDS-associated opportunistic infections, including viral pathogens, interactions among multiple pathogens and animal models of AIDS-associated opportunistic infections; Studies of HIV/AIDS-associated cancers, including animal models; Immunology of AIDS-associated opportunistic infections; and discovery/identification of therapeutic targets for AIDS-associated opportunistic infections.” The cover letter for any submitted grants should indicate how the proposed research relates to the mission of the AOIC study section. Discussions with NIH staff have indicated that basic biological studies of the classic opportunistic infections (such as pneumocystosis, microsporidiosis, and cryptosporidiosis) will continue to be reviewed in AOIC.

Roundtable discussion: Ibero-American Network about pneumocystosis

Dr. Enrique Calderón discussed the Ibero-American Programme for Science, Technology and Development (CYTED) which was created in 1984 through an International Framework Agreement signed by 19 Latin American countries, Spain, and Portugal (http://www.cytel.org/cytel_investigacion/detalle_accion.php)

un=4d5b995358e7798bc7e9d9db83c612a5&lang=es). The CYTED Program seeks to set up mechanisms for cooperation between research groups of universities, R+D centers, and innovative companies in Latin American countries. The “Red Iberoamericana sobre Pneumocystosis” (Pneumocystosis Ibero-American Network CYTED 212RT0450) is a Thematic Network supported by CYTED Program that was created in 2012. This Network involves 11 research groups from 8 countries (Chile, Venezuela, Cuba, Mexico, Brazil, France, Portugal, and Spain). The main objective of this network is the promotion of joint scientific research, the transfer of knowledge and practices, and the exchange of scientists and technicians mainly between member groups, but also with other groups in the field of *Pneumocystis* infection (www.iberopneumocystis.com).

CONTRIBUTED REPORTS

Microsporidia

The phylum Microsporidia comprises nearly 1,400 species of intracellular fungal parasites that infect vertebrate and invertebrate hosts of commercial, ecological, and medical significance. A unique feature of the Microsporidia is their mode of infection. During germination, the spore contents are propelled through an everted polar filament and injected into the host cell. Until a few decades ago, Microsporidia were important in agriculture and veterinary medicine, but with the growing AIDS pandemic, Microsporidia emerged as causes of opportunistic infections associated with diarrhea and systemic disease. With improved diagnostic methods and greater awareness, the number of Microsporidia species and hosts is growing. Microsporidia also are interesting for exhibiting gene reduction and compaction that are signs of extreme parasite efficiency. The reports highlighted some of the recent advances contributing to our understanding about the basic and applied biology of the Microsporidia. New species and hosts of infection continue to be identified, and the recent application of next generation sequencing (NGS) will usher in a new emphasis on linking genomics and proteomics for better understanding the evolution of these extraordinarily successful intracellular organisms. Such studies are expected to become applicable to identifying drug targets leading to effective treatments for agriculturally relevant hosts of economic impact (e.g. silk-worm, fish, shrimp, and birds) as well as for immune-deficient people infected with Microsporidia.

Resources and molecular biology—The Eukaryotic Pathogen Genome Database (<http://www.EuPathDB.org>) is a Bioinformatics Resource Center providing an online portal to complete genome sequences, annotations, and integrated function genomics data for multiple eukaryotes, including the Microsporidia [T2] and several of the species of Microsporidia infecting mammals and humans are available through the ATCC [T3]. Microsporidia possess the smallest genomes among the eukaryotes, which emphasizes their efficient adaptation to intracellular parasitism. Genomic studies revealed their remarkably reduced gene content and compaction, and yet, horizontal gene transfer and signatures of possible sexual reproduction such as meiosis-specific genes and heterozygosity exist in some species [T14]. Based on a set of orthologous genes identified in *Nematocida* spp. (infecting *Caenorhabditis elegans*) and Microsporidia genome sequences in various public databases, evolutionary events identified in the Microsporidia included gene loss, acquisition, and modification. Noteworthy was the loss of a tumor suppressor gene (Rb, Retinoblastoma) and acquisition of a secretion signal sequence for hexokinase, both of which probably modulate the host cell biosynthetic machinery to enable intracellular replication by these Microsporidia [T17]. Sequencing of additional species of Microsporidia infecting a wider range of hosts for comparative analyses will contribute to better understanding the success of these highly evolved parasites.

Cell biology and taxonomy—*Enterocytozoon bieneusi* is still considered the most common microsporidian species identified in humans and was the first species classified within the family Enterocytozoonidae. Genera of Microsporidia subsequently added to this family during the past 20 yr include *Nucleospora*, *Paranucleospora/Desmozoon*, *Hepatospora*, and *Enterosporea* and the combined application of ultrastructural features and molecular phylogeny based on SSUrDNA sequencing corroborated the monophyletic clade relationship among this group of organisms [T47]. Ultrastructural studies also were presented demonstrating that the multilayered interlaced network organelle especially recognized in *Anncalia algerae* sporoplasms during germination, exhibits enzymatic function and displays a complex series of interconnected flattened sacs, varicose tubules, and fenestrated flattened sheet-like structures reminiscent of Golgi cisternae [T43]. Whereas Microsporidia were long believed to lack mitochondria and Golgi, studies are now revealing that these organisms have minimized their organelles as represented by mitosomes and the multilayered interlaced network, respectively, to match their minimized genomes for achieving high efficiency as parasites.

Immunology—Microsporidia infections produce disease in immune-deficient mammalian hosts, but also cause persistent infections with few or no signs of disease in otherwise healthy immune-competent hosts. Adoptive transfer of intraepithelial lymphocytes comprised primarily of CD8⁺ T-cells that exert cytolytic activity, partially protected SCID mice after oral inoculation of *Encephalitozoon cuniculi*. Protection required trafficking of dendritic cells (DCs) to the mesenteric lymph nodes [T27]. Immune-competent BALB/c mice inoculated orally with *E. cuniculi* carried chronic infections detectable by PCR in many organs. Infections could be cleared by treatment with albendazole, but were reactivated if mice were immune-suppressed with dexamethasone [T32]. *Encephalitozoon cuniculi* and *Vittaforma corneae* are two species infecting humans, and both organisms were able to inhibit caspase-3 activity and gene expression as measures of apoptosis, in a human macrophage cell line [T30]. These findings demonstrate that host immune responses are needed to prevent lethal infections, but that the Microsporidia have evolved mechanisms to evade these responses sufficiently well enough to persist in immune-competent rodents, which could be predictive for the course of infection in humans.

Diagnostics, clinical observations, and epidemiology—A loop-mediated isothermal amplification method that could be applied in field studies was described for detecting *Nosema bombycis*, a cause of pebrine disease in silkworms, in China [T7]. In addition, a novel multiplex PCR assay and development of noninfectious molecular probes were described for discerning between *E. cuniculi* and *E. bieneusi* [PA9, PB6]. PCR-based methods continue to be applied for sensitive and specific identification of Microsporidia in tissue specimens and environmental sources to extend our knowledge about the epidemiology and potential sources of Microsporidia infections. HIV-infected patients with chronic diarrhea and weight loss in St. Petersburg, Russia were reported to have a higher prevalence of infection with *Encephalitozoon* species than *E. bieneusi*, the reverse of what has been reported elsewhere in the world [T38]. *Enterocytozoon bieneusi*, also, was especially prevalent among young HIV-infected and uninfected children under 2 yr of age in Nigeria [PB8, T57].

Reports were presented about Microsporidia species infecting humans that were identified in other hosts and environmental sources. *Encephalitozoon cuniculi* and *E. bieneusi* were reported in horses in the Czech Republic [PB11] and in free-ranging great apes across Africa [PB12]. The *Encephalitozoon* species and *E. bieneusi* were detected in free-ranging exotic birds and pigeons in Spain [PB14] and South American coatis [PB15]. *Enterocytozoon bieneusi* was identified in cattle concurrently infected with *Blastocystis*, *Cryptosporidium*, and *Giardia* [T55], and in free-ranging rhesus macaques in a public park in China [T56].

Drinking water and wastewater treatment plants in Spain were contaminated with *E. bienersi*, *E. cuniculi*, *E. intestinalis*, and *A. algerae*, thus possibly contributing to transmission of infection to humans and other hosts. In areas adjacent to the BP oil spill in the Gulf of Mexico, a higher rate of white Atlantic shrimp species was found to be infected with *Agmasoma penaei* that is associated with cotton disease.

Pneumocystis

Cell wall and glucan—The asci of *Pneumocystis* contain β -1,3-D-glucan (BG), while little to none is found in the trophic forms. Previous studies have shown that determination of the levels of BG in patients with fungal infections can discriminate *Pneumocystis jirovecii* pneumonia (PcP) from other fungal infections by the high amounts of this linear polymer found in the sera of PcP patients, which can range from several hundred to over 38,000 pg/ml. The mechanism of where these large amounts of BG originate and how they enter the bloodstream is currently not known. In a study of sera from 100 HIV-positive patients from Portugal collected from 1994 to 2000 and 20 noninfected donors, the median BG levels in PcP-documented cases using a commercial kit was 270 pg/ml, with a lactose dehydrogenase (LDH) median level of 707 U/L. In contrast, patients who were apparently colonized by *P. jirovecii* or were uninfected had median BG values of 45 pg/ml and LDH levels 420 U/L, while uninfected donors' median levels were 31.0 pg/ml and 141 U/L. In this study, both BG and LDH values could differentiate PcP patients from colonized or uninfected patients on a statistical basis [T5]. In contrast, in a smaller study of 11 lung transplant and 7 cystic fibrosis patients, the researchers could not differentiate colonized patients (as defined by a positive PCR directed to the rRNA ITS region) from noncolonized patients [T8]. Interleukin-8 is a potent chemoattractant that can activate neutrophils and other immune cells. In COPD patients colonized by *P. jirovecii*, there are high levels of circulating IL-8, which has been implicated in the pathophysiology of PcP. As airway epithelial cells can secrete IL-8, an effort to better understand the relationship between IL-8 release and the presence of BG in PcP + COPD patients was undertaken. Eleven PCR-defined *P. jirovecii* colonized patients were compared with noncolonized patients. Higher mean levels of IL-8 and BG were detected in the colonized patients (20.7 ± 19.7 vs. 8.9 ± 10.8 pg/ml, $p < 0.001$) and (44 ± 52.1 vs. 24.6 ± 27 pg/ml, $p = 0.565$), respectively [PA4], which did not indicate a causal relationship, but raises intriguing questions about the many roles that BG may play in the pathogenesis of PcP.

Although much of the emphasis is currently placed on the BG component of the *Pneumocystis* cell wall, it is likely that *Pneumocystis* contain other minor glucan components as is the case with other fungi. Studies focusing on β -1,6-glucan showed the presence of this immunostimulatory molecule in the asci cell walls by immunostaining [T42]. Homology cloning identified a functional β -1,6-glucan synthase (Kre6) in the *Pneumocystis carinii* genome, which could complement its yeast counterpart in a *kre6*-deficient yeast [T42]. This glucan was then biochemically isolated and when presented to macrophages on beads, a vigorous TNF- α response was observed. The inflammatory response could be dampened with PDMP, a glycosphingo-lipid lactosyl ceramide inhibitor, which serves as a glucan-binding molecule, implicating this glucan as a mediator of lung inflammation during PcP.

As the basic biology of *Pneumocystis* is slowly uncovered, a report describing the process of thigmotropism in response to contact with alveolar epithelial cells and lung matrix proteins provides insights into the mechanism that these fungi may use to infiltrate and spread among lung alveoli [T44]. Thigmotropism is defined as “the directional response, usually of a plant organ, to touch or make physical contact with a solid object. This directional response is generally caused by the induction of some pattern of differential growth” (p. 1 in Hart 1990).

The differential growth of *Pneumocystis* in this respect, involves the central regulatory protein PcSte20 MAP kinase, which phosphorylates the cell wall biosynthesis regulatory kinase, PcCbk1, which in turn stimulates the PcAce2 transcription factor that is a regulator of gene expression for cell wall biosynthetic and remodeling genes. Such studies underscore the close relationship that has evolved between this fungi and its mammalian host.

Drug development—There is a critical need for new and novel approaches to the treatment of PcP and potentially, those patients who are colonized by *P. jirovecii* or who have comorbidities associated with its presence. One such novel therapy was presented in the context of the identification of myeloid-derived suppressor cells (MDSC), which were reported to be abundant in the lungs of mice with PcP [T10, T11]. These cells are a heterogeneous population of hematopoietic precursors that can adversely affect host responses. Myeloid-derived suppressor cells can be characterized by expression of the markers, Gr-1, CD11b in mice, CD11bc and His-48 in rats. Adoptive transfers of these cells into immunologically normal mice cause lung damage, suggesting a role in the pathophysiology of PcP. Treatment with all-trans retinoic acid (ATRA) was used to push the cells to differentiate into functional macrophages [T11]. After 5 wk of treatment, the MDSC population was reduced and PcP cleared. The mice also realized increased survival and did not relapse. A combination of primaquine and ATRA equaled the standard TMP-SMX in terms of efficacy in reduction in fungal burdens. Such alternative treatments offer innovative means to combat the different manifestations of *Pneumocystis* infection.

Another unique approach to treatment of PcP was the use of IKT061, a drug in development from Inhibikase Therapeutics (Atlanta, GA), which targets the host cells and not the microbe [PA22]. In an ATP-driven bioluminescent in vitro assay system, the IC₅₀ was 1.22 µg/ml; moderate activity against *Pneumocystis*. PK/PD studies showed that the oral bioavailability of this compound was low (3–10%), but intraperitoneal dosing provided > 70%. In the mouse model of PcP, 20 and 50 mg/kg revealed lower organism burdens after 7 days of treatment, but this was lost after 14 and 21 days of treatment with burdens equaling those of untreated mice. Notably, those mice treated with IKT061 demonstrated a dose-dependent improvement of survival after 14 days of treatment; only 50% of the untreated mice were alive, while mice in both of the treated groups had 66% and 100% survival. This compound and its analogs provide a potentially new therapy for PcP, which may rely on stimulation of a host response rather than targeting the pathogen.

Chemotherapeutics has been the mainstay of anti-PcP therapy and the two standard therapies have been and still remain TMP-SMX and pentamidine isethionate. Pentamidine has been the parent compound of many chemical modifications to reduce toxicity and increase potency, as it is a monotherapy as opposed to the combination of TMP-SMX. In vitro screening using an ATP-driven bioluminescent assay of 20 pentamidine-parent modified compounds resulted in 5 having marked activity (IC₅₀ 0.1–0.99 µg/ml), 13 with moderate activity (IC₅₀ 1.0–9.99 µg/ml), and one with slight activity (IC₅₀ 10–49.99 µg/ml) [PA2]. Only one had no activity (> 50 µg/ml). In general, higher activity was associated with inclusion of a heteroatom in the aliphatic linker. Toxicity on the lung cell lines A549 (human) and L2 (rat) was only manifested by three of the compounds on the L2 cells, while none reduced viability of the A549 cell line. One such analog, PEN 10, was tested in vivo and found at 5 and 10 mg/kg to be as effective as 10 mg/kg pentamidine illustrating that the in vitro screening method was able to identify potential new anti-PcP therapies.

Previous studies to determine the utility of the new antifungal BG synthesis inhibitors, the echinocandins, showed that the ascus form was reduced to numbers undetectable by microscopic methods, but the trophic form numbers were largely left intact. In a follow-up study, a combination of low-dose TMP-SMX (12.25 mg/62.5 mg/day via oral gavage) and

casposfungin (0.1, 0.05, and 0.001 mg/kg daily) were administered for 21 days [PA8]. Results of *Pneumocystis murina* burden based on RT-quantitative PCR (qPCR) and microscopic enumeration showed that the combination of compounds, even at the lowest doses were more effective in lowering burdens than the compounds given alone at the doses noted above. Importantly, those mice given casposfungin alone never completely cleared the infection. As both treatments are available clinically, such combinations hold promise for new therapeutic intervention, especially with the inclusion of casposfungin, which reduced the glucan content, a pro-inflammatory factor that contributes to the pathophysiology of PcP.

Host responses—The host responses to colonization and pneumonia caused by *Pneumocystis* spp. are complex and a focus of the majority of research on these pathogens. Glucan is a known pro-inflammatory factor and is now understood to be a contributor to the detrimental immune response associated with PcP. The cell wall of the *Pneumocystis* asci contains abundant glucan, especially BG but also other minor components that can stimulate the immune responses like β -1,6-glucan. As treatment of mice and rats with echinocandins eliminates the asci, but not trophic forms, this chemical tool permits the dissection of the host responses to the two major forms of *Pneumocystis*, trophs and asci. In a mouse model of *P. murina* infection and immune reconstitution, a differential response was observed between mice that had asci in their infecting population (untreated) and those that had only trophic forms (anidulafungin-treated) [T26]. During the 2 wk of reconstitution, the presence of asci, which correlated with BG measurement, provoked a deleterious host inflammatory response that included a CD8⁺ T-cell influx, elevated TH17 markers, expression of macrophage inflammatory response markers, increased numbers of macrophages and neutrophils, which were associated with greater lung damage. There was a clear difference in responses to each of these populations, which has significant implications for clearance and on interpretation of the host immune response to what has generically been thought of as *Pneumocystis*.

TH cells regulate macrophage phenotype and effector functions. A TH1 signal induces a classical macrophage activation phenotype, while a TH2 is associated with alternative activation phenotype. Manipulation of the phenotype by the drug sulfasalazine (an NF κ -B inhibitor), to the alternative activation phenotype resulted in attenuated PcP-related immunopathogenesis in a mouse model of infection [T31]. Besides providing insights into the pathophysiological immune response, these results suggest that such modulation could be a unique therapeutic strategy.

The presence of *P. murina* in mice was also implicated in pathology located in the bone marrow besides damage to the lungs [T33, PA18]. Type I-IFNs are induced in lungs and serum of *P. murina*-infected WT and RAG^{-/-} mice. This response was abrogated in mice lacking the IFNAR-receptor IFN $\alpha\beta$ receptor (IFNAR). Lymphocyte competent IFNAR^{-/-} mice developed lung pathology and delayed clearance of the infection, and transient bone marrow depression with extramedullary hematopoiesis. Strikingly, mice lacking lymphocytes and IFNAR could not clear the infection and rapidly progressed to bone marrow failure and death due to apoptotic cell death. In addition, these mice were shown to have inflammation-induced accelerated osteoclastogenesis and bone loss. These results have serious implications for patients with impaired type I-IFNs, which can occur during HIV infection as well as steroid treatment.

Neonatal responses in mice to primary *P. murina* infection have been shown to be distinct from those of adult mice. The alveolar macrophages (AMs) are considered the first line of defense against these fungi. Neonatal AMs do not respond to *P. murina* infection with the same efficiency as that of adult mice [T29], which is related to the immunosuppressive lung

environment of neonatal mice, at least in part. Studies presented to dissect this defect [T28] demonstrated that neonates developed an alternative macrophage phenotype that was defined by the mannose receptor, Ym1 and arginase expression. IL-4, expressed by infiltrating CD4⁺ and neutrophils, appeared to drive this phenotype. On the other hand, deficiencies in IL-4 or IL-4R α resulted in faster clearance, leading to the possibility that the alternative AM phenotype in neonatal mice helps to maintain lung integrity within the developing lung.

In a study to translate host responses to diagnostic criteria, two markers, S-adenosyl methionine (SAM) and KL-6 were evaluated as serological markers [PA3]. Earlier studies reported the requirement of SAM for *Pneumocystis* growth, while *Krebs non den Lungen-6* (KL-6) is a mucin-like protein expressed on type II alveolar *Pneumocystis* and bronchial epithelial cells and is used as a marker of interstitial pneumonitis. Sera presenting with high levels of KL-6 (> 570 U/ml) were statistically associated with diagnosed PcP cases (77%, $p < 0.001$), while no significance was found for SAM.

Epidemiology and transmission—There is a growing body of evidence that *P. jirovecii* exploits populations of patients made susceptible by immunomodulatory agents or chronic diseases, such as COPD. The results of infection can range from colonization with or without effect, or can result in mortality. In a recent study from Spain [PA5], the prevalence of *P. jirovecii* colonization in patients receiving anti-TNF drugs (infliximab, adalimumab, or etanercept) was evaluated in 194 oropharyngeal washes using RT-PCR, from 63 controls with systemic diseases not receiving drugs, 62 patients receiving infliximab, 40 receiving etanercept and 30 receiving adalimumab with systemic diseases. *Pneumocystis jirovecii* was detected in 41 patients (21%) with no significant difference among the three treatments. In another study conducted at the Mayo Clinic, Rochester, using computerbased review, patients who received rituximab and developed PcP from 1998 to 2011 were analyzed [PA7]. Over this period, 30 patients developed PcP with an underlying disease of hematologic malignancies in 90% of the cases. Most used glucocorticoids (70%), but three patients (10%) developed PcP without adjunctive therapy. The clinical course was fatal in 30% of these patients, much higher than that for HIV-positive patients (10–15%). These studies indicate that such susceptible patients may require anti-*Pneumocystis* treatment.

Pneumocystis jirovecii has also been detected in acute interstitial pneumonia, a rare and rapidly fatal form of diffuse lung injury. The trigger of this rapid progression and the etiology of this disease are currently unknown, but it is suspected that an infectious disease may be the trigger. In five biopsy-confirmed cases collected in a Spanish hospital, the presence of *P. jirovecii* was detected in all by RT-PCR [PA6]. Two of the patients received TMP-SMX treatment and one of those was the only survivor of the five. Although there was no causal relationship established, the finding is intriguing and will need more study to determine causality.

Dihydropteroate synthase (DHPS) is the target of sulfonamides and the “SMX” in the TMP-SMX combination therapy. It has been known for over a decade that *P. jirovecii* has evolved mutations in the DHPS gene that led to increased resistance in other microbial pathogens, specifically at nucleotide 165 and 171, but a role for these mutations in clinical outcome has not been clear. A study was undertaken in the Brittany region of France to evaluate the prevalence of DHPS mutations [PA13]. Archival specimens from two hospitals were genotyped at the DHPS locus using an RFLP assay. Testing was successful in 63/84 patients. WT DHPS (no mutations) were detected in 62 of the 64 patients, while 2 had mutations. These two patients were from Paris and not Brittany. This low frequency of mutations was unusual compared to other centers in the area that were tested, for example, Paris had a 18.5–40% mutation prevalence.

A study to determine whether the DHPS genotype did have an effect on clinical outcome was conducted in France [T35]. Samples from 112 episodes of PcP in 110 HIV-infected patients were evaluated by PCR-single strand conformation of the DHPS gene. Death was the outcome in 18.8% episodes and attributable to PcP; 33% of the episodes were associated with DHPS with one or both mutations. Dihydropteroate synthase genotype had no impact on outcome within 1 mo, whereas genotype 7 and the need for mechanical ventilation were significantly associated with an increased risk of death due to PcP. Mechanical ventilation was also associated with risk of sulfa failure at 5 days. Another study conducted in the U.S. sought to identify predictors of clinical stability and relapse in HIV-associated PcP [T39]. At least one vital sign abnormality was seen in 93% of the study population of 475 hospitalized cases of HIV-associated PcP from 1999 to 2006. At most, 26% had abnormal values for all four vital signs at baseline. Factors associated with a slower time to clinical stability included a lack of PcP prophylaxis and more severe PcP. Relapse in any vital sign was associated with a longer hospital stay. Most patients improve within 2–4 days, but a relapse in fever, tachycardia, or tachypnea was associated with death.

Animal and human studies have provided evidence that that *Pneumocystis* is transmitted via an airborne route. More recently, technology has permitted the capture of exhalants from patients with PcP with subsequent genotyping as a means to track the transmission of specific genotypes on an individual patient basis [PA14]. In a study presented at the meeting, the air within the patients' environments (rooms) was tested for concordance with genotypes from patients' lung samples. Dihydropteroate synthase genotyping was successful for 15 pulmonary samples and 6 of 15 air samples. A full match of DHPS genotypes was found for six pairs of samples, providing evidence that the patients' genotypes were indeed those in the close environment. Such studies support more stringent measures for patient room sterilization. Patients who were hospitalized with PcP could disseminate *P. jirovecii* detectable by PCR. Another study using immune-competent rats inoculated with *P. carinii* and sampled for 60 days showed transient air excretion between days 14 and 22 of one experiment, and days 9 and 19 of the other [T49]. This was associated with a peak organism burden in the lungs, while IgM and IgG antibody increase preceded clearance of the fungi from the lungs and cessation of airborne excretion. These studies provide evidence that immune-competent hosts are able to excrete *P. carinii*, or at least DNA, after primary infection.

The studies on the transmission of *P. jirovecii* by patients detailed above [PA14] provides the rationale for previous outbreaks in sequestered areas and for more recent ones as described in renal transplant units from Germany, Switzerland, and Japan [T34]. In this multicountry study, RFLP was used to compare *P. jirovecii* isolates from these three outbreaks. It was shown that a single *P. jirovecii* genotype caused pneumonia in transplant patients in Switzerland (7 patients) and Germany (14 patients), but this genotype was distinct from the causal agent in the Japanese outbreak. In a study of Mozambican children less than 5 yr, 14% of the 835 pediatric patients with pneumonia over a 12-mo period were found to be positive for *P. jirovecii* [T37]. Three patients died, HIV-co-infection was present in 54.5%. This survey predicts a high prevalence among this population of children.

It has been suggested that each mammalian species harbors at least one species of *Pneumocystis*. *Pneumocystis* from tracheal washes of two foals diagnosed with PcP from a veterinary practice in Oklahoma were genotyped using four different genes [PB1]. There was not complete identify between the two genotypes, but they consistently claded together when compared with other *Pneumocystis* species. Of note, these sequences were distinct from a previously reported *Pneumocystis* sequence obtained from an English foal.

Genomics and genetics—With the advent of NGS, the genomes of uncultivable organisms like *Pneumocystis* can be sequenced, and their transcriptomes revealed during various phases of the infective process. The assembly of the human pathogen, *P. jirovecii*, was unveiled at the current meeting [T18] and has been released to the public: <http://www.ebi.ac.uk/ena/data/view/> (Cissé et al. 2012). Together with the newly released *P. murina* genome: <http://www.broadinstitute.org/annotation/genome/Pneumocystisgroup> and the *P. carinii* genome: <http://pgp.cchmc.org>, [PA21] these tools will now make it possible to evaluate synteny among these closely related fungal species; identify new and novel drug targets; and better understand the basic biology of these heretofore-enigmatic fungi. For example, it has been generally accepted that *Pneumocystis* utilizes host-derived cholesterol from its host, but what are the receptors for cholesterol and does it vary among the species? Why is *Pneumocystis* host-specific and what genes are involved in this specificity? In addition, a myriad of other metabolic questions can now be posed. The assembled genome of *P. jirovecii* currently is at 8.1 Mb, with a predicted gene inventory of 3,878, strikingly similar to predictions for *P. carinii*. A significant finding of the studies was the paucity of genes related to amino acid biosynthesis, which could provide clues to supplementation of in vitro media that could eventually lead to sustainable ex vivo growth. In short, the availability of genomic data promises to level the scientific playing field for these heretofore-intractable fungi.

However, without a sustainable in vitro system, investigators will still need to use heterologous expression systems to provide functional analysis of genes of interest. The library of *Saccharomyces cerevisiae* mutants available in the U.S. and in Europe has been a critical tool for these analyses. In most cases, *Pneumocystis*-derived genes from *P. carinii* have been successfully expressed in yeast. It will be important to see whether this will hold true for those from *P. jirovecii* and *P. murina*. Two such expression studies were reported at this meeting. In one, the S-adenosyl methionine:sterol C-24 methyltransferase (SAM:SMT), encoded by Pcerg6, was used to complement the yeast deletion strain and the sterol composition was measured [T12]. Ergosterol was the major sterol in strains complemented with the Pcerg6 and the Scerg6. C-28 and C-29 sterols were present, showing that the Pcerg6 enzyme was functional in yeast. However, the profile was distinct in the Pcerg6-complemented yeast from that of the WT yeast. This was likely due to the capability of the Pcerg6 to transfer one or two methyl groups vs. only a single methyl group by the Scerg6. *Pneumocystis* spp. are not known to synthesize ergosterol, the primary sterol of the vast majority of fungi, but rather scavenge cholesterol from the host. However, there is clear evidence that at least *P. carinii* has a functional sterol biosynthetic pathway that results in the production of lanosterol. Previously undetected in the fragmented genome assembly of *P. carinii*, a candidate for the Pcerg4 gene was recently identified [PA1]. Erg4 is the enzyme that catalyzes the final step of ergosterol biosynthesis in fungi. Complementation of the Pcerg4 in the yeast deletion strain resulted in no perceptible growth changes and in preliminary analyses, the presence of ergosterol in the complemented strain.

Cryptosporidium

Comparative genomics—Thus far, whole genome sequence data are available for only one isolate each of *Cryptosporidium parvum*, *Cryptosporidium hominis*, and *Cryptosporidium muris* genomes (Abrahamsen et al. 2004; Widmer and Sullivan 2012; Xu et al. 2004). In the era of NGS, routine whole genome sequencing of *Cryptosporidium* becomes feasible, although work in this area lags far behind other pathogens because of the lack of effective culture models for *Cryptosporidium* spp. The utility of NGS in studies of the pathogen biology of *Cryptosporidium* was demonstrated recently by comparison of the Illumina NGS data from an anthroponotic *C. parvum* subtype with the published whole genome sequence data from a zoonotic *C. parvum* subtype (Widmer et al. 2012). In a

presentation by Keely and associates [T15], a nearly complete genome (8.9×10^6 bp or 97.8% of the expected genome) of *Cryptosporidium meleagridis* was sequenced using the 454 technology, with an average sequence identity of 89.2% to *C. parvum*. More importantly, the researchers developed procedures for the whole genome sequencing of *Cryptosporidium* at the single oocyst level using a combination of flow cytometry sorting and whole genome amplification. Using similar procedures, another group sequenced the genome of *Cryptosporidium ubiquitum*, with 8.6×10^6 bp or 94.5% of the whole genome data obtained [T48]. The data were used to identify the gene coding for the 60-kDa glycoprotein (gp60) gene, which was used in the development of a subtyping tool for *C. ubiquitum*.

Biochemistry and host–parasite interactions—The availability of whole genome sequences has greatly facilitated research in biochemistry and host–parasite interactions of *Cryptosporidium* spp. Recently, the first oligonucleotide microarray for *Cryptosporidium*, CpArray15K was developed based on genomic sequences of *C. parvum* [T23]. It covered all predicted open reading frames in the parasite genome and was used in the study of transcriptome of oocysts. The oocysts were found to have highly active protein synthesis and high expression of proteasome, ubiquitin, and lactate dehydrogenase genes. Mining of the whole genome sequence database had led to the identification of three fatty acyl-CoA synthetase (ACS) genes, *CpACS1*, *CpACS2* and *CpACS3*, that might serve as novel drug targets, as this group of enzymes mediates the first step of reaction in fatty acid metabolism [T13]. *CpACS1* and *CpACS2* were expressed as recombinant proteins, and were shown to hydrolyze C10–C20 fatty acids and were expressed in all life cycle stages, implying their importance in the parasite development. The data mining had also led to the identification of a *C. parvum* protein containing a C-type lectin domain (CTLN), a signal peptide, a transmembrane domain, and a highly O-glycosylated mucin domain [PA11]. The CTLN protein was successfully expressed in *T. gondii*. As proteins containing CTLNs are known to mediate diverse cell–cell interactions in higher eukaryotes, this new *C. parvum* protein could play a potential role in *Cryptosporidium*-host cell interactions.

The role of DCs in immune responses to *C. parvum* infection was assessed in a mouse model [PA16]. Dendritic cells could be activated by *C. parvum* antigen to produce cytokines such as IL-12 p40, IL-2, IL-1 β , IL-6, and IL18. These responses were MyD88-dependent. Mice depleted of DCs were highly susceptible to *C. parvum*. In another study, profiles of cytokines and immunoglobulins in *C. parvum* were examined using the Luminex[®] xMAP technology (EMD Millipore Corporation, Billerica, MA) [PA20]. A preferential TH1 response and predominance of IgG1 and IgG2a were seen *C. parvum*-infected mice.

Molecular epidemiology—Molecular epidemiology of cryptosporidiosis is an area of active research and was well represented in IWOP-2012 presentations. This is largely because *Cryptosporidium* spp. are commonly found in humans, animals, and environment, as shown in presentations on their prevalence in humans in India [PB9] and Nigeria [PB8]; cattle in India [PB9], Romania [PB5]), and the U.S. [T55]; sheep in Algeria [PB16]; pigs in the Czech Republic; nonhuman primates in Africa [PB12]) and China [T56]; rodents in the Czech and Slovak Republics; birds in Algeria [T51]; and water in Spain [PB5]. Because most *Cryptosporidium* spp. are morphologically similar, molecular diagnostic tools are used increasingly in the identification of infection sources in humans and assessment of the public health significance of parasites in animals and environment (Xiao 2010). As a result, commercial molecular standards were established as positive controls in PCR analysis of common *Cryptosporidium* species [PB6]. The analytic sensitivity of PCR for *Cryptosporidium* oocysts in water samples was estimated using the Most Probable Number approach [T6].

Several studies have assessed the distribution and public health significance of *Cryptosporidium* species in animals using genotyping tools. In one study by a research group in the Czech Republic, several unusual *Cryptosporidium* species, including *C. bovis*, *C. muris*, and *C. meleagridis* were found among 250 chimpanzees and gorillas examined in Africa (PB12). A number of different *Cryptosporidium* spp. was also found in 55 of 364 fecal samples from 8 rodent species of the family Muridae the Czech and Slovak Republics in a second study by the same group of investigators (PB13). The most common *Cryptosporidium* species were *C. muris* (14 samples) and *C. ubiquitum* (12 samples), which were found in most rodent species. Other species included *C. parvum* (2), *Cryptosporidium canis* (2), *Cryptosporidium andersoni* (1), *Cryptosporidium tyzzeri* (1), *Cryptosporidium muskrat* genotype II (2), *Cryptosporidium vole* genotype (1), *Cryptosporidium* SW1 genotype (1), and 13 novel *Cryptosporidium* genotypes (in 19 samples). Likewise, two host-adapted *Cryptosporidium* species, *C. suis* and *C. scrofarum*, were identified in 355 *Cryptosporidium*-positive samples from 1,620 pigs examined in the Czech Republic (T54). Only one sample had *C. parvum* and three had *C. muris*. *Cryptosporidium suis* was primarily detected in piglets (preweaned), while *C. scrofarum* was only detected in starters, especially those that were 4 wk old. Pigs raised on straw bedding were more likely to have *Cryptosporidium* than pigs raised on slats/slurry systems. The difference in age distribution of the two species in pigs was attributed to the difference in age-specific infectivity of the parasites (PB10). Experimental infection revealed susceptibility of both 4- and 8-wk-old pigs to *C. suis*, but only the 8-wk-old pigs to *C. scrofarum*. Although they are occasionally found in pigs in field settings, *C. muris* and *C. tyzzeri* were not infectious to pigs in experimental infections. Surprisingly, only *C. andersoni* (4/7) and *C. muris* (2/7) were identified, in 7 of 40 samples taken from five natural swimming pools in Spain during a 1-yr period (PB2).

In contrast, human-pathogenic species were the dominant *Cryptosporidium* in two studies in animals in close contact with humans. PCR-RFLP analysis of 90 ileum samples from sick or dead chickens and turkeys in Algeria identified *Cryptosporidium* in 43 (46.7%) samples, with *C. meleagridis* in all positive turkeys and most chickens, and *C. baileyi* in five chickens (T51). The *C. meleagridis* identified belonged to a new subtype family based on sequence analysis of the gp60 gene. In a study conducted in free-range rhesus monkeys in a large public park in China, 45 (10.9%) of 411 fecal samples were positive for *Cryptosporidium* by PCR (T56). The anthroponotic *C. hominis* was the dominant species, being found in 39 fecal samples and belonging to six subtypes. *Cryptosporidium parvum* was found in five samples, all belonging to the anthroponotic IIc subtype family. *Cryptosporidium felis* was found in only one fecal sample. The same *C. hominis* subtypes were found in 11 of 23 water samples from a small lake in which the monkeys routinely bathed.

In several studies, the role of animals in the transmission of human cryptosporidiosis was examined by genotyping and subtyping *Cryptosporidium* spp. in fecal samples collected from humans and animals living in the same area. In one study [PB9], fecal samples from 180 cattle and 51 farmers on two dairy farms in India were analyzed by PCR-RFLP. *Cryptosporidium parvum*, *C. bovis*, *Cryptosporidium ryanae*, *C. andersoni*, and a *C. suis*-like genotype were found in 11.7% of positive cattle, with the four common species following the well-established age-related pattern in *Cryptosporidium* species distribution in dairy cattle (Xiao 2010). In contrast, three, two, and one farmer(s) were infected with *C. hominis*, *C. parvum*, and a novel *C. bovis* genotype, respectively. In another study in Romania, only *C. parvum* was identified in 15 *Cryptosporidium*-positive samples from cattle and three from humans [PB5]. Unlike in other European countries where IIa subtypes were the dominant *C. parvum* in cattle and humans, most of the *C. parvum* found in this study belonged to four subtypes in the IIc subtype family. The likely occurrence of zoonotic transmission of cryptosporidiosis was also examined in a subtyping study of *C. ubiquitum*

[T48]. Sequence analysis of the gp60 gene in 53 human and animal isolates led to the identification of four subtype families, with one major subtype family in domestic and wild ruminants in the Old and New World, and three subtype families in wild rodents in the U.S. All four subtype families were detected in humans living in the same areas as the animals. Domestication of sheep was postulated to be a major factor in the spread of the ruminant subtype family around the world.

FREE-LIVING AMOEBAE, GIARDIA, AND KINETOPLASTIDS

A number of reports centered on epidemiological studies that were undertaken in several countries to determine the presence of pathogens in the environment and their relationship with infection in humans and animals. Many of these studies demonstrated the transmission of multiple organisms in the studied populations. The transmission of *Pneumocystis* by the airborne route and close contact was reported for humans and experimental animals [T1, (Walzer, PD; PA14, T49)]. Sprague–Dawley rats were utilized to demonstrate that *Pneumocystis* could be released into the air by immune-competent hosts [T49]. Sheep farming has been shown to be an important factor in the spread of *C. ubiquitum*, an emerging zoonotic pathogen [T48]. In Algeria, studies were undertaken to determine the presence of *C. meleagridis*, in chickens and turkeys. These studies highlighted the zoonotic potential of this species as 40% of chickens and 50% of turkeys harbor this parasite [PB16]. Fecal samples from swine were examined for the presence of *C. suis* and *Cryptosporidium* pig genotype II, in the Czech Republic. *Cryptosporidium suis* was identified by PCR in samples from 16 of 22 farms. Husbandry practices were found to be important in the infection process in swine [PB10]. Dairy cattle were examined in the U.S. for *Blastocystis* spp., *Cryptosporidium* spp., *Giardia duodenalis*, and *E. bieneusi* by PCR. Fayer et al. found that 3- to 5-mo-old cattle were infected concurrently with three to four of these parasites [T55]. In a study in Romania, it was [PB5] reported that 41% of cattle were infected with *C. parvum*. In India, studies by Ganguly suggested a potential risk of zoonotic transmission of *Cryptosporidium* spp. and *G. duodenalis* between cattle and humans on dairy farms [PB9]. A study in China [T56] reported that nonhuman primates, examined in a public park frequented by humans, were infected with anthroponotic enteric parasites including *C. hominis*, *G. duodenalis*, and *E. bieneusi*. The authors suggested that these animals served as a reservoir of human pathogens. Studies [PB8, T57] in Nigeria suggest that anthroponotic transmission may be important in *Cryptosporidium* spp. and in *G. duodenalis* infections, while *E. bieneusi* infection occurs more often due to zoonotic transmission to humans. Studies in Brazil [PB14] determined that stool specimens from a number of exotic birds including parakeets, parrots, and pigeons, were positive for *Encephalitozoon* and *Enterocytozoon* spores. In addition, these authors also studied the occurrence of Microsporidia in the South American coatis [PB15]. The authors emphasized the importance of birds, pigeons, and the coatis as potential sources of Microsporidia infections for humans living in large cities. Studies on the presence of pathogens in recreational and domestic water supplies and the environment were reported, also. An approved method (USEPA Method 1623) available for the detection of pathogens in drinking water is time consuming and does not distinguish pathogenic from non-pathogenic organisms. A most probable number PCR method was described that combines a statistical and molecular approach to improve detection of the usual waterborne pathogens, *Giardia* and *Cryptosporidium*, in drinking water [T6]. An improved standard for PCR of environmental samples for *Giardia*, *Cryptosporidium*, and Microsporidia was described [PB6]. *Giardia* spp. and *Cryptosporidium* spp. were detected in recreational water in Spain [PB2]. In addition, these authors also reported the presence of pathogenic Microsporidia and *Cyclospora* from several drinking water treatment plants, providing evidence that drinking water is a potential source of infection of these pathogens in Spain [PB3].

Two other waterborne pathogens, *Acanthamoeba* spp. and *Naegleria fowleri*, are free-living amoebae that can cause fatal CNS infections in humans. Recreational water such as fresh water lakes and ponds, improperly chlorinated swimming pools, and the domestic water supply are sources of infection. A survey was conducted in Tehran Province, Iran, to determine the occurrence of these amoebae. Pathogenic *Acanthamoeba* and *Naegleria* were identified using PCR [T52]. *Acanthamoeba* spp. also can cause a sight-threatening infection, amoebic keratitis, which is more common in contact lens wearers than in the general population. However, in Spain, although a high incidence of *Acanthamoeba* in tap water has been reported, the occurrence of amoebic keratitis in contact lens wearers is low [PB4]. In other countries, such as the U.K. and the U.S., the incidence of amoebic keratitis is higher. The authors suggested that hygiene habits such as rinsing lenses with tap water and wearing contact lenses while showering might be important factors in the incidence of corneal infection with *Acanthamoeba*.

One group presented a series of studies examining the gene expression and cell cycle in isolates of *Giardia intestinalis* [T16]. The cell cycle studies used counterflow centrifugal elutriation (CCE) to obtain fractions of *Giardia* trophozoites for cell cycle studies. Quantitative PCR analysis of CCE fractions enriched in S-phase cells showed an increase in *Giardia* histone mRNAs, and fractions enriched in G2/M cells showed an increase in mRNAs from putative cyclin-B and cyclin-B-like genes. Studies of the gene expression among different *Giardia* assemblages (A–G) demonstrated differentially expressed genes [T16] consistent with the differences in the ability of these assemblages to infect different species and the ability of different isolates of the same assemblages to cause differences in the severity and length of giardiasis.

TOXOPLASMA

Infection by *T. gondii* in healthy individuals usually results in minor symptoms, but can cause death or defects in brain development in a fetus carried by pregnant mothers lacking immunity to this parasite. Immune-deficient adults are also at risk for toxoplasmosis, and can develop life-threatening encephalitis. It is now appreciated that infection may have effects on the behavior of the host, even when the infection is asymptomatic or latent [T36]. While new drugs are needed for acute infection, there are no current therapeutic agents that treat latent infection and eliminate tissue cysts. Transmission of *T. gondii* is due to ingestion of bradyzoites (tissue cysts) within meat or via the ingestion of oocysts, which can survive for long periods in the environment, in food or water. Endochin-like quinolones were screened for *T. gondii* activity and several were parasitocidal [T27, PA10] suggesting that they may be useful for latent infection. Ciprofloxacin derivatives were also demonstrated by another research group to have activity against *T. gondii* and caused morphological changes in the apicoplast [PA19]. A series of phage display libraries [PA15] and monoclonal libraries to tachyzoite surface proteins [PA17] were developed against *T. gondii* and clones from these libraries are being evaluated as diagnostic reagents.

Molecular biology

Several *T. gondii* strains have been sequenced and www.ToxoDB.org now has complete annotated genomes for type I–III strains along with corresponding epigenomic, transcriptomic, and proteomic data [T2]. There is significant interest in the histone code of this organism and how it relates to the regulation of genes and the development of latency. A JmjC1 histone demethylase has been characterized and found to be essential for parasite survival [T20]. JmcC1 appears to have a repressive role on inactive genes and maintains the heterochromatic regions of the chromosome [T20]. The histone code of this organism was determined by a mass spectrometry (proteomics)-based approach [T21] demonstrating conserved as well as unique posttranslational modifications on the various *T. gondii*

histones. Analysis of genomic data has identified *T. gondii* proteins that can target the host cell nucleus and affect epigenetic regulation of host genes [T22]. RNAseq analysis of the transcriptomes of RH (a type I strain), P (a type II strain) and CTG (a type III strain) at pH 7 and pH 8 demonstrates strain-specific gene expression as well as gene expression related to differentiation [T24]. Notably, several genes involved in differentiation were either chromatin modifiers or AP2 family members, suggesting that epigenetic regulation is important in stage differentiation.

Cell biology

Nitric oxide is important in the host response to *T. gondii*. Suppression of nitric oxide is a survival mechanism for this pathogen. A mutagenesis approach was described that identified 12 clones that are impaired for survival in activated macrophages and all 12 clones were unable to stand nitrosative stress suggesting a defect in this pathway [T25]. Characterization of these mutants is in progress. Another mechanism by which host cells eliminate infection is via interferon-regulated genes of which immunity related GTPases (IRGs) have been demonstrated to be critical regulators of infection in nonimmune cells. These IRGs lyse the parasitophorous vacuole and virulent strains of *T. gondii* (type I strains) block this process by phosphorylation of these IRGs [T41]. Using fluorescent-labeled parasites, it was found that parasites could escape vacuoles to which IRGs were targeted [T41]. Using monoclonal antibodies and proteomic techniques, the proteins making up the tissue cyst wall (bradyzoite parasitophorous vacuole membrane) were characterized [T45]. One of these proteins was knocked out in *T. gondii* PRU-KU80 and was found to be important for stabilization of the cyst wall, i.e. the knockout resulted in fragile cysts [T45]. Studies on glycosylation in *T. gondii* tissue cysts using host cells with glycosylation defects has demonstrated that *T. gondii* tissue cyst wall proteins undergo posttranslational N-glycan modifications due to host cell rather than *T. gondii* enzymes [T46]. A new strain of *T. gondii* named EGS isolated in Brazil demonstrated a high rate of spontaneous cystogenesis in vitro in epithelial cells (LLC-MK2) with the development of thick cyst walls [PA12].

IWOP-13

At IWOP-11, it was decided that the IWOP meetings will alternate between North America and Europe every 2 yr. To this end, the next International Workshops on Opportunistic Protists will be in Spain in 2014. Information on IWOP-13 is available at <http://iwop2014.atlantacongress.org/> or <http://www.iwopmeeting.org>. The local organizing committee is chaired by Enrique J. Calderon ecalderon@ibis-sevilla.es.

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