



ISID NEWS

An Official Publication of the International Society for Infectious Diseases

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ISID NEWS

Editorial Staff

Amy Galblum
Jaylyn Olivo
Nancy Voynow
Paul Guttry

181 Longwood Avenue
Boston, MA 02115-5804 USA
Telephone: (617) 277-0551
1 (800) 779-8998
Fax: (617) 731-1541
E-mail: info@isid.org
<http://www.isid.org>

ISID and TheraSim

The ISID was one of the first organizations to develop a sustained HIV clinical training program for physicians from low resource areas. Since 1998, the Society has enabled more than 120 physicians from approximately 50 low resource countries to participate in a comprehensive educational program based at recognized international centers of excellence including the National Institutes of Health, Memorial Sloan Kettering Cancer Center, and San Francisco General Hospital. The goal of this program has always been to create training opportunities for those who need to be trained, not just those who can afford to be trained.



The ISID recognizes that the needs of the global HIV/AIDS pandemic require new approaches if the workforce essential for achieving the WHO clinical capacity building goals, which calls for increasing by the number of HIV-trained health providers and community treatment supporters worldwide by 100,000, are to be met. To this end, ISID and TheraSim have developed a groundbreaking initiative to transform current educational approaches for training health care workers in HIV/AIDS clinical care and to substantially

expand the number of physicians and related health care workers skilled in the prevention, diagnosis, and treatment of HIV/AIDS. The Society's Global HIV Training Initiative uses new educational technologies developed by TheraSim, Inc. to create a comprehensive patient simulator for training clinicians in the diagnosis and treatment of infectious diseases such as HIV and HIV co-morbidities.

TheraSim is a medical education company started in 2003 by David Hadden, an early pioneer of clinical decision support systems for infectious disease. TheraSim uses expert systems technology to create a fully interactive clinical simulation environment where a clinician can meet a patient, order labs, make his or her diagnosis and develop a treatment path. The system provides the user with instant clinical feedback on items such as drug interactions, toxicity, resistance, therapy staging and other clinical issues. The system's therapy simulation engine produces this feedback by measuring the fitness of the proposed therapy for that patient against a knowledgebase of best practices, which includes pharmacology data, prescribing information and guidelines. The system then provides clinical feedback to the learner. The system also tracks clinical competency and skill levels over time. When skill gaps are identified, the system prescribes didactic simulations and practice simulations to help the learner gain additional competency in that area. The didactic material is drawn from the ISID's HIV clinical training program as well as guidelines such as the WHO's Integrated Management of Adult and Adolescent Illness (IMAI).

With the help of our partners at leading medical institutions, the Society has been training physicians from many different countries in the effective treatment of HIV for years. Though this program is very successful, the traditional training methods we employ are not able to expand the cadre of HIV-ready clinicians at the pace required to meet the demands of the epidemic on an international scale. To meet the global need for qualified HIV care providers in the near future, it is essential that new training tools and methods be developed.

Electronic-based training programs have the potential to dramatically increase the number of clinicians who may be trained at the same time and with substantially less cost. The TheraSim diagnostic and treatment simulation software is an exciting opportunity to set a training standard in HIV heretofore unattainable on a large scale.

— Tim Brewer MD, MPH • ISID Program Director

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The program has been dubbed the ISID-TheraSim Virtual Clinical Training Program (VCTP). The VCTP took its first steps with a classroom based exposure of the application to 50 African physicians in the Spring of 2004 and in February of 2005; the program became available in a pilot mode on the ISID website and was announced on ProMed Mail. The response was astonishing, with physicians from over 56 countries signing up. In the first 6 weeks, 274 physicians used the program and their feedback was better than 97% positive.

The system tracks issues related to clinical knowledge of treatment guidelines and is able to produce reports that show deviations from guidelines. Among the early findings, participants failed to order ART and PCP prophylaxis in 48% and 74% of the cases despite clear indications. Candida esophagitis and *Herpes zoster* infections were not treated with medications 82% and 69% of the time, while indicated HIV markers (CD4 count and viral load) and resistance assays were not obtained in 58% and 54% of cases. Of note, overall dosing errors occurred in 42% (d4T and ddI were most commonly associated with errors—38% and 37% of the time). Finally, ineffective regimens based on available resistance testing were ordered for 18% of the regimens.

ISID is uniquely positioned to develop and support a technology assisted comprehensive international HIV/AIDS training program. The Society has the institutional relationships, program management experience, and global credibility necessary to undertake this essential educational endeavor and to succeed. Our International HIV Advisory Board includes some of the most respected HIV clinician-scientists in the world. The ISID and TheraSim are currently in discussion with potential partners to scale this program globally. ❖

TheraSim[®]
Reach More. Teach More. Save More.

The 12th ICID in Lisbon

Planning continues for the 12th International Congress on Infectious Diseases to be held in Lisbon, Portugal from June 15–18, 2006. In addition to the three exciting plenary speakers highlighted in the last issue of the ISID NEWS, the Congress will be host to a plenary address by Dr. Bonnie Bassler, a 2002 MacArthur Foundation Fellow, and currently a Professor of Molecular Biology at Princeton University, and the Director of Graduate Studies in the Molecular Biology department. The research in Dr. Bassler's laboratory focuses on the molecular mechanisms used by bacteria for intercellular communication, a process called quorum sensing. She received the New Jersey Thomas A. Edison Patent Award for Medical Technology in 2003 and the New York Intellectual Property Lawyer's Association chose her as the 2004 Inventor of the Year Technology in 2003. Her talk is titled, "Bacterial Crosstalk: Implications for Pathogenesis And Treatment."



Bonnie Bassler

PLENARY LECTURES

Bonnie BASSLER, United States

Bacterial Crosstalk: Implications for Pathogenesis And Treatment

Edward FEIL, United Kingdom

Bacterial Microevolution: Relevance to the Clinician

Antoni TORRES, Spain

Severe Community-acquired Pneumonia—A Genetic Predisposition?

Bernhard SCHWARTLÄNDER, Switzerland

The Impact of the Global Fund to Fight AIDS, Tuberculosis and Malaria after Five Years

Confirmed Speakers as of May 24, 2005

AMARAL, Leonard (PORTUGAL)
ANDERSEN, Peter (DENMARK)
AYLWARD, Bruce (SWITZERLAND)
BARRY, Clifton (USA)
BASSLER, Bonnie (USA)
CERCENADO, Emilia (SPAIN)
COHEN, Jon (UNITED KINGDOM)
CRUBEZY, Eric (FRANCE)
DAGAN, Ron (ISRAEL)
DE LENCASTRE, Herminia (PORTUGAL)
DRANCOURT, Michel (FRANCE)
DUSE, Adriano (SOUTH AFRICA)
EREMIN, Sergei (RUSSIA)
ETIENNE, Jerome (FRANCE)
FARRELL, David (UNITED KINGDOM)
FEDSON, David (FRANCE)
GAVALDA, Joan (SPAIN)
GOLENOCK, Douglas (USA)
GRAYBILL, John (USA)
HILL, Adrian (UNITED KINGDOM)
JOHNSTON, Margaret (USA)
KLUGMAN, Keith (USA)
KNOBEL, Hernando (SPAIN)
LILJESTROM, Peter (SWEDEN)
LOW, Don (CANADA)
LUCET, Jean-Christophe (FRANCE)
MAERTENS, Johan (BELGIUM)
MIRA, Jean-Paul (FRANCE)
MIRO, Jose Maria (SPAIN)
MULLIGAN, Connie (USA)
MUMCUOGLU, Kosta (ISRAEL)
O'BRIEN, Kate (USA)
O'RYAN, Miguel (CHILE)
ODIO, Carla (COSTA RICA)
PINEDA, Juan Antonio (SPAIN)
SARMENTO E CASTRO, Rui
(PORTUGAL)
SCHRENZEL, Jacques (SWITZERLAND)
SPRUNG, Charles (ISRAEL)
STOHR, Klaus (SWITZERLAND)
STONE, Sheldon (UNITED KINGDOM)
TAUBENBERGER, Jeffery (USA)
TEMMERMAN, Marleen (BELGIUM)
TURNIDGE, John (AUSTRALIA)
VALADAS, Emilia (PORTUGAL)
WHITE, Nicholas (THAILAND)
WOLF, Michel (FRANCE)

12th International Congress on Infectious Diseases • Symposia

June 15–18, 2006 • Lisbon, Portugal

The following symposia are being organized ~ Updated May 24, 2005.

New Developments in Institutional MRSA Control

- Worldwide MRSA Spread: The Bridge Between Molecular Biology and Healthcare
- Evidence: What Works, What does not Work for MRSA Control?
- Successful Control of Endemic MRSA: Is it Possible?
- Rapid Screening for MRSA: Myth or Reality?

Antibiotic-Resistant *S. pneumoniae*: Antibiotic Restriction and Pneumococcal Vaccines

- Do PD/PK Parameters Predict Emerging Resistance?
- New Antibiotics and New Antibiotic-resistance Mechanisms in pneumococci
- What Have We Learned from Trends in Antibiotic Resistance After Introduction of PCVs?
- Do Interventions in Antibiotic Use Influence Antibiotic-resistant *S. pneumoniae*?

Septic Shock

- Innate Immunity: Friend or Foe?
- Are All Hosts Equal? Do Genetics Matter?
- Antibiotic Choice in Sepsis: A Forgotten Art
- Steroids Good or Bad—A Critical Appraisal

Modern Identification of the Outbreaks of the Past

- Plague and Black Death
- Spanish Influenza
- Mycobacterium Disease at the Origins of Urban Life in Egypt
- Human Louse Infestations: Past and Present
- Pre-Columbian Syphilis

Pandemic Influenza: Are We Ready Yet?

- The Molecular Virology of Influenza Vaccines and Its Implications for the Next Pandemic
- Pandemic Influenza Preparedness at the National and International Level
- Prevention and Control of the Next Pandemic with Vaccines and Antivirals: Prospects for an Equitable Global Supply
- The Political Dimensions of the Next Influenza Pandemic: A Threat to Global Health Security

New Directions in Malaria Treatment

- How PK/PD Characteristics and Resistance Affect Treatment Strategies
- Assessing Treatment Efficacy in Vivo: What Should We Follow?
- Treating Resistant Malaria in Pregnancy
- New Antimalarial Agents and New Therapeutic Combinations

Preventing the Big Three: Vaccines for Malaria, TB, and HIV

- Cell-mediated Immunity: Challenges for Developing Effective Vaccines
- Update on Malaria Vaccines: In the Field and the Laboratory
- Tuberculosis Vaccines: Ready for Prime Time?
- A Modern Odyssey: HIV Vaccine Development After 20 Years

Tuberculosis and HIV Infection

- Treatment of Tuberculosis in HIV-infected Patients
- Treatment of Latent Tuberculosis Infection in HIV-infected Patients
- Strategies to Improve Patient Adherence to Tuberculosis Treatment
- Infection Control and Treatment of Latent Infection in Patients with Multidrug-resistant Tuberculosis

Update on Pneumococcal Vaccines

- Update on PCV Effect on Pneumoniae and Invasive Infections (US, Europe, Latin America)
- The Replacement Phenomenon in PCV—What are the Implications?
- Pneumococcal Protein-based Vaccines: The Next-Generation Pneumococcal Vaccines?
- Global Pediatric Pneumococcal Vaccination Implementation: Where Are We in 2006?

Challenges in Infection Control in Low-Resource Countries

- Quality Improvement in Infection Control in Resource Limited Setting
- Surveillance for the Detection and Control of Multiple Drug Resistant Nosocomial Pathogens in the Middle East
- Establishing Effective Control Programs for Surgical Site Infections
- Dealing With Formidable and Novel Pathogens in Minimally-resourced Settings

Optimizing Care for Invasive Fungal Infections

- Diagnosing Fungal Infections: What Test Strategies Improve Outcomes?
- Fungal Infections in the ICU: When and What Empiric Therapy?
- Pro: Is Combination Anti-Fungal Therapy the New Standard for Invasive Fungal Infections
- Con: Is Combination Anti-Fungal Therapy The New Standard for Invasive Fungal Infections

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12th International Congress on Infectious Diseases • Symposia

June 15–18, 2006 • Lisbon, Portugal

The following symposia are being organized ~ Updated May 24, 2005.

Vaccines for Children: New Strategies

- Present and Future of Rotavirus Vaccines
- Hepatitis A: Experiences with Universal Immunization
- Polio: Defining the Optimal Vaccine Strategy
- Vaccination for Premature Infants: What Vaccines When?

Tuberculosis: Past, Present and Future

- Genetic Evolution of Tuberculosis: Co-Travelers in Global Migration
- Treating Tuberculosis in HIV-infected Patients in a Low-Resource World
- Tuberculosis Drug Development: Where Are We in 2006?
- Tuberculosis Control Beyond DOTS: What Do We Know?

HIV Treatment Challenges in High- and Low-Resource Settings

Advances in Pediatric Infectious Diseases

Hepatitis

Interactive Clinical Cases

Advances in Microbiology: From the Bench to the Clinical Laboratory

The Role of Women in Infectious Diseases

- The Current Role of Women in Families in the Global Prevention and Control of Infections
- Women as High-Risk Victims of Infection
- Women as Leaders in the Effort to Control Infections
- The Relation Between Women's Rights and Infectious Diseases

Beyond Institutions: MRSA in the Community

- Clonal Epidemiology Worldwide
- Prevalence and Risk Factors
- Treatment Options for Community-acquired Staphylococcal Infections: End of Beta-lactam Era?
- Control Strategies



The ICID Preliminary Program is now available online at http://www.isid.org/12th_icid/

ISID would like to acknowledge the following

COLLABORATING AND COOPERATING ORGANIZATIONS

12th International Congress on Infectious Diseases

Lisbon, Portugal • June 15–18, 2006

Collaborating Organizations:

Portuguese Society of Infectious Diseases (SPDI)

Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC)

Cooperating Organizations:

Association of Medical Microbiologists (United-Kingdom)

Brazilian Society of Infectious Diseases (SBI)

Croatian Society of Infectious Diseases

Danish Society for Clinical Microbiology (DSKM)

European Society of Clinical Microbiology and Infectious Diseases (ESCMID)

Finnish Society for Clinical Microbiology

French Infectious Diseases Society (SPILF)

German Society of Infectious Diseases

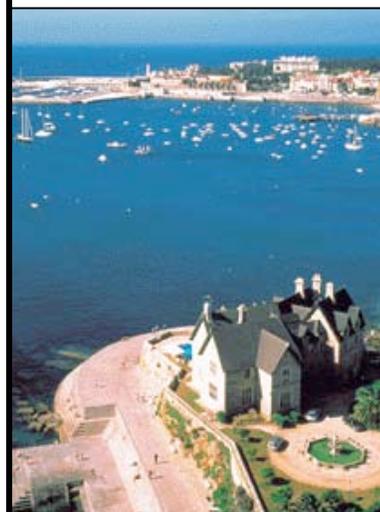
Hellenic Society for Infectious Diseases

Norwegian Society of Medical Microbiology

Pan American Association of Infectious Diseases (API)

Swiss Society for Infectious Diseases

Turkish Microbiological Society (TMC)





Dr. Laura Rodero



Dr. Rodero is the head of a Laboratory at the Mycology Department of the National Institute of Infectious Diseases (INEI, ANLIS, Dr. Malbran) Buenos Aires, Argentina. She has been working since 1994 in antifungal susceptibility of pathogenic yeasts, especially in *Candida albicans* and *Cryptococcus neoformans*.

ISID Small Grants Program Final Report

by Dr. Laura Rodero, Laboratory Head • National Institute of Infectious Diseases • Buenos Aires • Argentina

Amino acid substitutions of 14- β demethylase (ERG11) in fluconazole resistant *Cryptococcus neoformans* clinical isolates

Background: Cryptococcal meningitis or meningoencephalitis is the most frequent manifestation of infections due to *Cryptococcus neoformans*. Treatment of patients with AIDS can be complicated by severe immunosuppression. The very high relapse rate after standard regimens of amphotericin B has led to the use of lifelong chronic suppressive therapy. Though the current recommendation for maintenance treatment is oral fluconazole, its long-term usage for fungal infections has been documented in several studies as contributing to azole resistance. Paugam *et al.* and Friese *et al.* have reported cases of recurrent cryptococcosis during chronic suppressive therapy. Sequential isolates from these patients were found to have increased levels of fluconazole MICs.

Mechanisms of azole resistance in yeasts already described include altered affinity of the 14- β lanosterol demethylase (ERG11) to azole drugs due to target site mutation or its overexpression, and decreased accumulation of drugs due to enhanced energy-dependent drug efflux. The biochemical basis of fluconazole resistance in *C. neoformans* has been explored, especially regarding the reduction of azole cellular content and the altered activity of ERG11. Changes in azole affinity for ERG11 have already been related to low-level fluconazole resistance in *C. neoformans* isolates. In addition, the decreased affinity of ERG11 for azole derivatives, due to mutations that increase levels of MICs of fluconazole, has been described in sequential clinical isolates of *Candida albicans*.

To determine whether this mechanism could also be implicated in *C. neoformans* azole resistance, we compared the ERG11 genomic sequence in five sequential isolates recovered from recurrent episodes of cryptococcal meningitis. Isolates 1–4 were fluconazole-susceptible and the fifth had a MIC of 16 μ g/ml. PCR amplification and sequencing of the gene encoding 14- β lanosterol demethylase showed a point mutation responsible for the amino acid substitution G484S in the resistant strain only.

Aim: In order to detect any point mutations in this gene associated with amino acid substitutions of 14- β lanosterol demethylase as a mechanism of azole resistance, we studied the sequence of gene ERG11 in clinical fluconazole-resistant *C. neoformans* isolates.

Methodology: In order to select *C. neoformans* (CN) isolates with MICs \geq 16 mg/l to fluconazole (FCZ), we evaluated the in vitro susceptibility against fluconazole of 200 isolates of from HIV patients with cryptococcal meningitis and stored in the culture collection of the Mycology Department, INEI, ANLIS: Dr. Malbrán.

CN strains from 25 patients were selected to study the gene ERG11. CN ATCC 90112 and 2 strains previously reported with and without an amino acid substitution G484S in the 14- β lanosterol demethylase were used as control strains (CN-1, without mutation and CN-5, with the ERG11 point mutation).

Results: Fragments of 2,147 bp containing the full sequence of ERG11 gene of all the isolates selected were obtained by amplification of DNA with a specific set of primers. From all the isolates tested, only CN strains isolated from two patients showed a single point mutation at the 1855 nucleotide of the ERG11 gene. The strains with this mutation correspond to Patient 3 (3rd Episode) and Patient 25 (2nd and 3rd Episodes): this point mutation was previously described and results in the substitution of amino acid glycine 484 for serine (G484S) in the deduced protein sequence of 14- β lanosterol demethylase. The G484 is a residue that forms part of the conserved hemo-binding domain and is conserved in all cytochrome P450 ERG11/Cyp51 of yeasts and filamentous fungi. In all cases, FCZ MICs of these mutated isolates were \geq 16 mg/l as the CN-5 control strain with the same point ERG11 mutation (1). In Patient 3 this mutation was absent in the previous isolate obtained during the 2nd episode (P-3), showing a FCZ MIC of 4 mg/l. Unfortunately, in case of patient 25, the only strains available at our laboratory and at the Hospital were those from episode number 2 and 3. In both, the mutation was detected, and they were highly resistant to FCZ (MIC of 32 mg/l and 128 mg/l, respectively). To verify that this point mutation was not due to errors introduced by the PCR amplification, the ERG11 gene from these mutated isolates was newly amplified and sequenced a second time and the point mutations confirmed.

Discussion: The results showed that strains of *C. neoformans* isolated from only 2 of the 25 patients studied had a point mutation. Surprisingly, both had the same amino acid substitution in the 14- β lanosterol demethylase (ERG11) enzyme (G484S), which coincides with the one previously reported for *C. albicans*. The authors remarked that most of these substitutions were present in enzyme domains highly conserved across yeasts and filamentous fungi. Four amino acid substitutions (D116K, K128T, E266D, and G464S) were more frequent, but only G464S was observed in resistant strains. In the *C. neoformans* isolates from these two patients it was also clear that the high MIC of FCZ was associated with the G484S amino acid substitution. However, due to the low frequency of mutation, the involvement of another concomitant molecular mechanism of resistance is quite possible. ♦

ISID Small Grants Program Final Report

by By Dr. Hongjie Liu, Assistant Professor

Wayne State University School of Medicine • Detroit, Michigan • USA

A pilot study of HIV counseling to increase willingness to receive premarital HIV testing among marriage license applicants in a rural area of China

The HIV epidemic in China is growing rapidly. A unique feature of the HIV epidemic in China is that the majority (80%) of HIV-infected persons are rural residents. The origin of the epidemic among drug users and plasma/blood donors in rural areas probably explains the unusual epidemiology of HIV in China. With the support of ISID, we conducted a study among marriage license applicants in a rural area of China, where HIV spread among former commercial plasma donors. The objectives of the study were (1) to examine the risks of HIV transmission between husbands and wives and from parents to children; (2) to document relationships among HIV-related public and felt stigma, worry of HIV infection, HIV/AIDS knowledge and disclosure of HIV testing results; (3) to assess the impact of HIV counseling on willingness to receive HIV tests and reducing the stigma associated with HIV. A one-group pretest-posttest study with one session of HIV counseling was conducted among 605 (302 couples) marriage license applicants in 2003.

HIV-related risks exist among the study population, and there is the potential that HIV could be transmitted to both spouses and children

More males (64.6%) than females (52.1%) reported having had premarital sex and multiple sex partners (12.6% and 6.9%, respectively). Among those having had multiple sex partners, 8.5% reported often or always using condoms. Couples had a low level of HIV knowledge and perception of vulnerability to HIV infection. Thirty-nine percent of the couples (119/302) knew that HIV could be transmitted within HIV-discordant couples, and 41% knew that HIV could be vertically transmitted from a mother to a child. Only 36.8% of the couples agreed that they would not plan to have a baby after knowing they were HIV-infected. About 43% of the couples agreed that they would use condoms consistently if one of them were HIV-positive. Multivariate analysis indicated that subjects were more likely to accept condom use if they (1) received a high-school education or above, (2) felt that they knew their spouse very well, (3) had greater HIV knowledge, (4) did not plan to have a baby if a spouse was infected, or (5) had had premarital sex. The finding of risk of HIV transmission among the marriage license applicants underscores the need for national programs to prevent HIV infection within couples, especially in rural areas.

Concern about HIV-related stigma is prevalent, and public stigma and felt stigma co-exist among rural marriage license applicants

Stigma can be conceptualized as either external (public stigma) or personal (felt stigma). Public stigma consists of the attitudes or reactions of the general population towards persons with HIV and their family members. Felt stigma, on the other hand, refers to an individual's fear of societal attitudes and potential discrimination if they were to have HIV infection. This study demonstrates that concern over HIV-related stigma was prevalent among the study population. The following relationships showed statistical significance: (1) HIV/AIDS knowledge \square perceived worry about HIV infection ($\square = -0.39$); (2) perceived worry \square public stigma ($\square = 0.27$); (3) public stigma \square felt stigma ($\square = 0.22$); and (4) felt stigma \square willingness to disclose HIV positive result ($\square = -0.20$). HIV-related public stigma and felt stigma co-existed, which makes efforts to reduce HIV-related stigma more difficult, because intervention programs need to target both public stigma and felt stigma at both the individual and community levels.

Impact of HIV counseling on willingness to receive HIV test and HIV-related stigma reduction

After the single session of HIV counseling, the level of HIV/AIDS knowledge increased (from a mean value of 6.77 to 11.46 out of a total score of 13), and the level of worry about HIV infection was decreased (from 13.66 to 8.87, total score: 24). The vast majority of individuals (80%) reported willingness to voluntarily receive an HIV test. It is feasible that voluntary counseling and testing programs for HIV (HIV-VCTs) can be implemented among this population in rural areas. However, the changes in perceived public stigma, felt stigma, and the willingness to disclose HIV-positive status were of marginal significance. Therefore, HIV counseling reduced perceived worry but exerted little impact on HIV-related stigma and disclosure. This lack of effect may be because the single-session counseling intervention was effective largely at the individual level, while HIV-related stigma and willingness to disclose are determined at both the individual and the community level.

Another finding from the study is that both temporary rural-to-urban migrants and non-migrants were at elevated risk of contracting and transmitting HIV, via different mechanisms. It is more practical to establish HIV intervention programs targeting rural residents before they leave for cities for a temporary job. \diamond



Dr. Hongjie Liu



Dr. Hongjie Liu completed a doctoral degree in Epidemiology at the School of Public Health, University of California, Los Angeles (UCLA). Before coming to UCLA, he worked as an epidemiologist at Anhui Provincial Center for Diseases Control and Prevention (CDC) for 12 years. He has published 38 peer-reviewed papers. Dr. Liu was elected a member of the U.S. National Public Health Honorary Society and is currently an assistant professor at Wayne State University School of Medicine.



Alemka Markotic, MD, PhD



Dr. Alemka Markotic is a Researcher at the Institute of Immunology, Zagreb, Croatia and the Associate Professor at the Medical Faculty University of Rijeka, Croatia. Her group is focused on research of innate immunity to intracellular pathogens especially to hantaviruses and is mostly supported by the Croatian Ministry of Science, Education and Sport.

We are grateful to the ISID for supporting this study. Ljiljana Cebalo, MD, Institute Immunology of Zagreb, Croatia performed most of the experiments, and Connie Schmaljohn, PhD, US Army Medical Research Institute of Infectious Diseases (USAMRIID), Frederick, MD, USA served as a consultant.

ISID Small Grants Program Final Report

by Alemka Markotic, MD, PhD, Associate Professor • Cellular Immunology Unit
Department for Research and Development, Institute of Immunology • Zagreb • Croatia

Mechanisms of Apoptosis in 293HEK cells infected with Hantaviruses

The genus *Hantavirus* (family *Bunyaviridae*) is responsible for two different syndromes: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS), both important problems in global public health. Following the incubation period, HFRS is manifested mainly by fever, variable degrees of circulatory failure, hemorrhage, and renal failure, but symptoms demonstrating the involvement of other organs and systems are also observed. In HPS there is initially a febrile phase associated with myalgia, headache, and malaise followed by progressive pulmonary edema with hypotension and hypoxemia.

Hantaviruses are maintained in nature in persistently infected rodents and can also persistently infect cultured mammalian cells, causing little or no cytopathology. During our studies of persistence, we unexpectedly observed cytopathic effects (CPE) and apoptosis in hantavirus-infected human embryonic kidney cells (HEK293). Our initial results (A. Markotic *et al.*, J Gen Virol 2003) indicated that members of the TNF receptor superfamily did not contribute to the apoptosis that we saw in the infected HEK293 cells.

To better understand kidney immunopathology in HFRS/HPS and the mechanisms of viral persistence, it would be of great importance to identify the apoptosis pathways and factors that lead to cytopathology. We looked for the expression of some TNF ligands and TNF receptors that were not tested in our previous study, bcl-2, caspases, IAP, TRAF, CARD, death domain family members, death effector domain family members, CIDE domain family members, as well as genes involved in the p53 and ATM pathways. To investigate the possible role of adhesion molecules and extracellular matrix proteins, which may be involved in cytopathogenicity during the interaction of HFRS- and HPS-causing hantaviruses and 293HEK cells, we looked for the expression of various types of cell adhesion molecules (such as the integrins, IgG superfamily members, cadherins and catenins, and selectins) as well as extracellular matrix proteins, proteases (such as the matrix metalloproteinases and the serine and cysteine proteinases) and their inhibitors.

We used Focused Gene Expression cDNA Array Analysis (GEArrays, SuperArray Bioscience, Frederick, MD, USA) to compare expression profiles of genes in RNA samples from HEK293 cells infected with Hantaan virus, Andes virus, and Sin Nombre virus. Two different GEArray™ were used: GEArray Q series Human Apoptosis Gene Array and GEArray Q series Human Extracellular Matrix & Adhesion Molecules Gene Array. Selected genes were identified by RT-PCR to verify transcriptional responses.

Increases in gene expression of p53, BCL2, BCL2-interacting killer (BIK), and caspase 7 in infected cells indicated that one of the mechanisms of apoptosis might involve the release of apoptotic factors from the mitochondria. Changes were also detected in cysteine cathepsins, which are implicated in a multitude of physiological and pathophysiological processes: degradation of extracellular matrix, apoptosis, and events of inflammatory and immune responses etc. A lysosomal pathway, characterized by partial rupture of lysosomal membranes, may be engaged simultaneously with mitochondrial permeabilization and caspase activation. The changes in gene expression for alpha (v) beta3 integrin (vitronectin receptor, receptor for pathogenic hantaviruses) and “bridging” thrombospondin (TSP), together with several metalloproteinases (MMP1, MMP2, MMP3, MMP13, and MMP24) and their inhibitors, which are critical modulators of extracellular matrix (ECM), may be involved in apoptosis. Metalloproteinases are known specifically to degrade ECM components such as fibronectin, vitronectin, and laminin.

Our findings at the gene expression level indicated that the mechanisms of apoptosis in HEK293 cells induced by hantaviruses are the result of the complex interplay among various molecules.

We have presented evidence that one of the mechanisms of apoptosis may involve the release of factors from the mitochondria. It is also possible that lysosomal rupture precedes and is necessary for the activation of the mitochondrial pathway of cell death.

Additionally, several important adhesion and extracellular matrix molecules may play roles in hantavirus-induced apoptosis in HEK293 cells. We also would like to highlight the importance of matrix metalloproteinases and their inhibitors, whose role in the immunopathogenesis of hantaviruses is shown here for the first time.

Further in vitro experiments should confirm the mechanisms of apoptosis at the gene expression level. Additionally, some of our findings direct us towards further clinical studies among HFRS and HPS patients, especially focused on kidney disorders.

An understanding of the mechanisms leading to apoptosis in HEK293 cells infected with HFRS- and HPS-causing viruses may yield to improved approaches to control the immunopathogenesis of kidney disorders, especially in HFRS. Additionally, it may also improve our understanding of the mechanisms of the persistence and pathogenesis of hantaviral infections in general. ❖

ISID Fellowship Program Final Report

by Ikechukwu Okoli, MSc, Assistant Lecturer

Department of Applied Microbiology, Nnamdi Azikiwe University • Awka, Anambra State • Nigeria

Molecular characterization of virulence factors of five isolates of *Cryptotrichosporon anacardiense* (CBS 9549, CBS 9550, CBS 9551, CBS 9552 and CBS 9553), a new basidiomyceteous yeast genus from Nigeria

The human pathogenic yeast *Cryptococcus neoformans* is known to be associated with many tree species. In June of 2002, I began an ecological investigation into the environmental sources of *C. neoformans* in Nigeria. I recovered some yeast colonies that later yielded 5 different isolates of a new encapsulated basidiomyceteous yeast species. The isolates were from the fresh flowers of the cashew tree (*Anacardium occidentale*) from Nnobi in Anambra State, Nigeria. This yeast formed pale brown colonies on Niger seed agar. This was a very interesting discovery, because the yeast also phenotypically resembled *C. neoformans* by growing at 37°C, expressing a polysaccharide capsule, and forming melanin pigments with exogenous phenolic substrates. These three characteristics are important virulence factors of *C. neoformans*.

With the support of ISID, the Albert Einstein College of Medicine (AECOM, New York, USA), and in collaboration with other workers at the National Institutes of Health (Maryland,

USA), and Centraalbureau voor Schimmelcultures (CBS, the Netherlands), we were able to establish morphological, biochemical, and molecular biological characteristics of this new yeast isolate. We were also able to generate phylogenetic trees based on the ITS region and the D1/D2 domains of the 26S rDNA from the Nigerian isolates, which enabled our taxonomic placement of the yeast among the trichosporonales, where the 5 isolates formed a basal lineage cluster. Our work on the yeast is being peer-reviewed for publication. As the yeast could not be identified with any known genus or species, we proposed the name *Cryptotrichosporon anacardii* gen. nov. et sp. nov.

The skills I acquired at AECOM include DNA isolation, polymerase chain reaction (PCR), nucleic acid sequencing, and molecular techniques for characterization of virulence factors. These skills will not only assist me in the completion of my PhD program, but will become an invaluable asset to my research career as a whole. ❖



Ikechukwu Okoli, MSc



Ikechukwu Okoli completed his BS and MS at Nnamdi Azikiwe University, Awka, Nigeria, where he is currently an Assistant Lecturer and a PhD student at the Department of Applied Microbiology. His major interests are the epidemiology and ecology of pathogenic fungi in Nigeria.



Gabriella Gago

Small Grant Fall 2004 Awardees

Gabriella Gago

Instituto de Biología Celular y Molecular de Rosario, Departamento de Microbiología, Argentina

Project: Molecular, genetic and biochemical characterization of Acyl-CoA carboxylase complexes of *Mycobacterium tuberculosis*: finding new drug targets for treating mycobacterial diseases

Hasan Bin Hamza

Department of Family Medicine, Aga Khan University, Pakistan

Project: Hepatitis survey in adults of Jam Kandah, Landhi District, Karachi, Pakistan

Maria Papathanasopoulos

Department of Molecular Medicine and Haematology, University of the Witwatersrand Medical School, South Africa

Project: Nonprogressive HIV-1 subtype C infection—viral determinants

ISID Fellowship 2005 Awardees

Maria Eugenia Alvarez • Argentina

TB Center, Public Health Research Institute, International Center for Public Health, New Jersey, USA

Project: Characterization of hosp response during *Mycobacterium tuberculosis* infection by gene expression profiling

Varsha Atul Potdar • India

Centres for Infection, Health Protection Agency, London, UK

Project: Application of sensitive methodologies for detection of transmitted HIV drug resistance



Hasan Bin Hamza



Maria Papathanasopoulos

Larry Madoff, MD
Editor, ProMED-mail

Updates from ProMED-mail

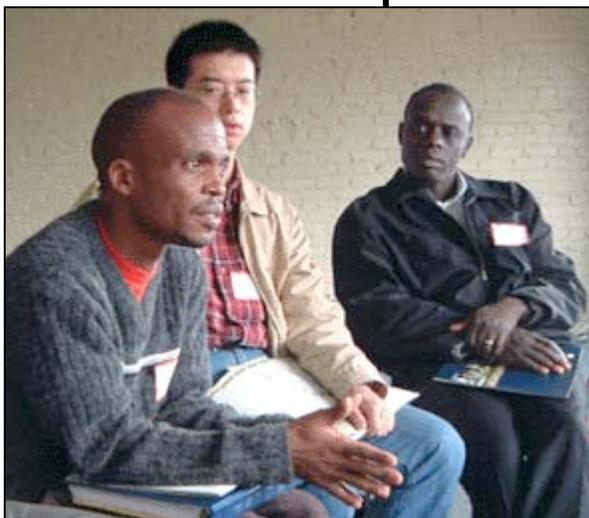
Some estimates place viruses as the cause of 70% of emerging infectious diseases. ProMED-mail's reporting thus far in 2005 certainly supports this contention. Avian influenza reporting has dominated the emerging disease landscape this year, with over 130 reports on ProMED-mail as of late May. Since reappearing in 2003, the H5N1 influenza has led to the culling of millions of poultry throughout Southeast Asia. The human caseload has remained under 100, with 53 deaths, most of them in Viet Nam. While only one confirmed incident of human-to-human transmission has occurred, the public health community widely fears that evolution of the virus will lead to greater contagiousness and the eventual birth of a global human pandemic.

The world's largest-ever outbreak of Marburg hemorrhagic fever has proved difficult to contain in Uige, Angola. With over 300 cases and greater than 90% case-fatality rate, the war-ravaged health care resources of this region have been overwhelmed, but successful isolation and control appears to be taking hold in the third month of the outbreak. Marburg's more familiar viral relative, Ebola, flared in the nearby Republic of Congo in May 2005 with 11 suspected cases and 9 deaths. This and other outbreaks of Ebola have been linked to consumption of monkey meat although the exact reservoirs of the virus remain uncertain.

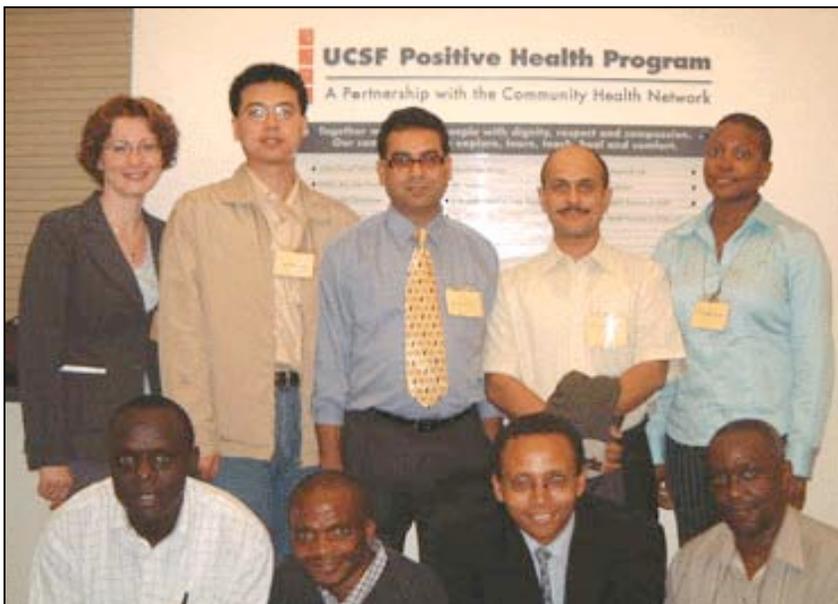
These epidemics, as well as the ongoing emergence of Dengue, West Nile Virus, Yellow fever and myriad other viral pathogens, consume a large share of ProMED's time and resources. Our monitoring of these emerging viral diseases has been greatly enhanced by the recent addition to our staff of a second viral disease moderator, Dr. Robert C. A. Yang. Recently retired from the National Health Research Institutes (NHRI) in Taipei, where he held the post of Professor of Infectious Diseases, Dr. Yang has worked in the field of emerging and re-emerging infectious viral diseases (e.g., HIV/SIV, STLV and dengue fever) for the past 15 years in Gabon, Uganda, Chad, Canada and Taiwan. Prior to that, he had significant hands-on experience on molecular study of several DNA tumor viruses (e.g. SV40 and BKV) for approximately 2 decades. We are happy to welcome Dr. Yang, who will join Dr. Craig Pringle in providing insightful commentary to ProMED's viral disease reporting. ❖

2005 ISID HIV/AIDS Training Program

The annual 2 week long HIV/AIDS Training Program educates clinicians from developing and transitional countries in clinical, therapeutic, epidemiological and public health issues. Sixteen participants from 12 countries attended the course that was held this year at three sites: the National Institutes of Health in Bethesda, Maryland; Memorial Sloan-Kettering Cancer Center in New York City; and University California at San Francisco. ❖



UCSF Left to right: Prosper Lutala, Yanbin Liu, Alex Muganzi.



UCSF Back: Justyna Kowalska, Yanbin Liu, Nirmal Rimal, Sumit Kane, Nadine Johnson. Front: Alex Muganzi, Prosper Lutala, Zenebe Melaku, Phineas Gitta (guest).