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THE MICRO-SCOOP

Newsletter of the University of Maryland School of Medicine
Department of Microbiology & Immunology

SCIENCE IN THE PUBLIC INTEREST

Vaccine Development

By: James B. Kaper, PhD

Vaccination is considered to be the most effective medical intervention in history. The first vaccine was developed more than 200 years ago by Edward Jenner, who hypothesized that milkmaids were spared the ravages of smallpox because of the mild cowpox infection that they acquired from the cows they milked. In 1796, he undertook a rigorous test of this hypothesis by actively immunizing an 8-year old boy with cowpox and later challenging him with smallpox—an experiment that would never be approved by today's Institutional Review Boards (IRBs)! Since that first gutsy experiment, vaccines developed against diphtheria, pertussis, tetanus, polio and other diseases have been responsible for enormous decreases of morbidity and mortality due to infectious diseases.

Over the past two decades, one of the research projects in my laboratory has been the development of a live attenuated vaccine for the prevention of the severe diarrheal disease cholera. Originally the NIH Recombinant DNA Guidelines prohibited the cloning of the *ctx* genes encoding cholera toxin, but as soon as the ban was lifted, we proceeded to clone and sequence these genes. Starting with a virulent *Vibrio cholerae* strain, we constructed mutants that were deleted of the genes encoding both the A and B subunits or just the enzymatically active A subunit only. These strains were tested in volunteers by my clinical colleagues in the Center for Vaccine Development (CVD) of the University of Maryland and were the first recombinant bacterial vaccines to be administered to humans. We were surprised to find that although the first vaccine candidates conferred strong protective immunity to disease and did not cause cholera, they nevertheless still caused mild diarrhea in about half the subjects. We have spent many years investigating the cause of this reactogenicity and developed several other vaccine candidates that were mutated in genes encoding other potential toxins. We eventually developed a vaccine candidate, called CVD 103-HgR, that is safe and well tolerated. The safety of this vaccine has been documented in studies involving over 70,000 subjects in North America, South America, Europe, Africa, and Asia, including infants and HIV-positive adults. The vaccine confers protective immunity against cholera as assessed in challenge studies in which volunteers ingest a wild type, fully virulent *V. cholerae* strain. In such studies, a single dose of the vaccine confers greater than 90% protection against moderate or severe diarrhea due to wild type *V. cholerae*. (continued on Page 2)

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Our cholera vaccine is now manufactured by Berna Biotech in Switzerland under the trade name Orochol and is licensed for sale in numerous countries around the world. However, we are still not certain of the reason for the reactogenicity seen with our early vaccine candidates. CVD 103-HgR colonizes the intestine less efficiently than the other strains, although interaction with the intestinal M cells is sufficient to confer a strong protective immune response. We believe that these earlier strains stimulated an inflammatory response in the intestine that is responsible for the reactogenicity, and we are currently collaborating with Dr. Stefanie Vogel (a fellow grad student of mine at UMCP) in characterizing these inflammatory mechanisms. In addition, we are using CVD 103-HgR as a vector vaccine to express heterologous antigens from other pathogens including rotavirus and enterohemorrhagic *E. coli* O157:H7 with the goal of developing vaccines against these pathogens.

The field of vaccine development has seen tremendous technical advances over the last two decades, particularly in molecular aspects. The use of recombinant DNA techniques has led to efficient production of cloned antigens, the development of novel attenuated vaccines, the use of bacterial and viral vectors to express heterologous antigens, the development of DNA vaccines (also called genetic immunization), the delivery of antigens through transgenic plants, and the discovery of new antigens via genomic approaches including "reverse vaccinology," wherein the genomic sequence of a pathogen is used to predict potential vaccine candidates that are then cloned and expressed. This same time period has also seen dramatic advances in our understanding of the immune system and our ability to manipulate this system to enhance antigen presentation and processing. These advances include the development of new adjuvants, the linkage of innate to acquired immunity, the characterization of lymphokines and other immunomodulators, and improved responses to T-independent antigens via conjugate vaccines. Indeed, current limitations and problems in vaccine development are rarely due to technical constraints but are usually due to an incomplete understanding of the biological system, including aspects of both the host and the pathogen. Our experience with the cholera vaccine certainly illustrates this fact.

Since September 11, 2001, tremendous resources have been dedicated to the development of new and improved vaccines against agents of bioterror, including anthrax and smallpox. The CVD is the lead institution for the Mid-Atlantic Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research, which is a consortium of sixteen research institutions dedicated to developing and testing such vaccines. In my own lab, we are currently investigating the use of our attenuated *V. cholerae* vaccine as a vector for the expression of the anthrax Protective Antigen (PA) and other antigens of bioterror agents. Although we would otherwise welcome the infusion of new funds into vaccine development, the need for such vaccines is distressing. The worldwide eradication of smallpox through vaccination is undoubtedly one of the greatest accomplishments of medicine. What an incredible tragedy it would be if this triumph were to be reversed by a few zealous individuals in pursuit of their political agenda.

Catch the "Yellow Fever"

Join our department's intramural soccer team, **Yellow Fever**. We will be playing teams from the other programs/schools here on campus. The games will be played on Sunday mornings from September 26 through mid-November at the soccer fields at Western High School on Falls Road (by Cold Spring Lane).

SPOTLIGHT ON NEW STUDENTS

Nicole Ammerman is a native of Portage, Wisconsin and received a dual BS at the University of Wisconsin, Madison in the diverse fields of Medical Microbiology and Immunology and Scandinavian Studies. Upon graduation, Nicole relocated to Baltimore and obtained her Master's degree from the Johns Hopkins University School of Public Health in Infectious Disease Epidemiology. For her Master's thesis she worked with Dr. Doug Norris, conducting a cross-sectional study investigating the prevalence of spotted-fever group Rickettsia in Maryland *Dermacentor variabilis*.

Smita Chandran was born in Mumbai, India and received both her Bachelor's and Master's in Life Sciences from St. Xavier's College, University of Bombay. Her research interests lie in the field of immunological tolerance and autoimmunity. In her spare time she enjoys watching movies and reading fiction.

Andy Holzgrefe is originally from Richmond, Virginia and graduated from the University of Virginia in 2000 with a BA in Biology and from the Virginia Commonwealth University in 2004 with a Master's degree in Public Health. His growing interest in microbiology was greatly boosted by the 1995 Ebola outbreak in Kikwit, Zaire. He has worked at the Virginia State Public Health Lab in the West Nile virus surveillance program and at the Virginia Commonwealth University in a molecular parasitology lab studying *Trypanosoma cruzi* and *Streptococcus sanguis*. His past, present and future interest is virology and its intersection with public health. When Andy is not doing schoolwork or sleeping he tends to indulge his passion for British history.

Priyanka Karicherla was born in Hyderabad, India and earned both her Bachelor's in Microbiology and Master's in Biotechnology from Osmania University in Andrha Pradesh, India. Her past research experience includes work at the Indian Institute of Chemical Technology which focused on isolating novel secondary metabolites from fungi and testing for the anti-microbial activity of these metabolites. Her interest in bacterial pathogenesis and vaccine biology brought her to UMB. In her spare time she enjoys making and listening to classical music as well playing sports, embarking on long drives and gazing at the night sky!

Mark Saltis was born in Philadelphia, Pennsylvania and attended the University of South Florida where he received a BS in Ichthyology and Marine Biology. After graduation, he moved to Maryland and worked at Martek Biosciences in the Protein Structure division. He later worked at Gene Logic in Gaithersburg, Maryland conducting pre-clinical research in Immunology and Pharmacology. Mark is a USCG-licensed captain and spends most of his free time on the Chesapeake fishing, crabbing and boating.

Khandra Sears was born in Nassau, Bahamas and obtained her BS in Microbiology from the University of the West Indies in Cave Hill, Barbados. She got her first taste of scientific research during her undergraduate

years, where she studied the microbial flora of Hawksbill turtle eggs. Upon graduation, she moved with her family to Washington, D.C. and worked as a volunteer research assistant in the Department of Immunology at the American Red Cross Holland Labs in Rockville, Maryland. At the Red Cross, her main project focus was trying to understand the mechanism of action for manumycin-A, a farneslytransferase inhibitor, in the induction of apoptosis in immature B cell lymphomas. Her hobbies include reading and playing volleyball. She recently joined Toastmasters to work on her public speaking skills.

Minjun Yu received his Bachelor's degree from the Beijing Medical University and did research at the Peking University Stem Cell Research Center that focused on stem cell development and differentiation. He is interested in the role that intracellular signaling pathways play in both development and immunological defense mechanisms. Aside from his research interests, he enjoys reading and traveling (although he has yet to purchase a car) and is particularly interested in Chinese history and archaeology. Unfortunately, he was unable to bring his collection of Chinese ancient dynasty coins with him when he moved to the United States.

MEETINGS, TRAVEL & AWARDS

Dr. Stefanie Vogel has been elected to Fellowship in the American Academy of Microbiology. An honorific leadership group, the American Academy of Microbiology recognizes excellence, originality, and creativity in all sub-specialties of the microbiological sciences. She will be recognized for this distinction at the Academy Fellows Luncheon and Meeting at the 104th ASM General Meeting in New Orleans, LA, on Wednesday, May 26. 2004.

Three of the six intramural grants submitted by our faculty have been funded in the amount of $15,000 for the period 7/1/04-6/30/05. These were awarded to **Drs. Ricardo Feldman**, **Ferenc Livak**, and **Yuko Ota**.

Dr. James Kaper, Professor of Microbiology & Immunology and Associate Director for Laboratory Research of the Center for Vaccine Development, has recieved a MERIT award (R37) from the National Institute of Allergy and Infectious Diseases (NIAID) of the NIH. The MERIT (Method to Extend Research In Time) award is intended to "provide long-term stable support to investigators whose research competence and productivity are distinctly superior and who are likely to continue to perform in an outstanding manner" (NIH Guide for Grants & Contracts). Dr. Kaper earned this MERIT award through his RO1 research grant entitled "Molecular Genetics of Enteropathogenic *E. coli* Adhesion". He has repeatedly renewed this grant for the past 19 years. It was recently renewed for another four years (years 19-23) and was converted to the R37 MERIT award at the NIAID Council meeting in May, 2004. The MERIT Award will allow this grant to be extended for an additional 5 years of support (years 24 – 28) without having to go through a competitive renewal review in a study section. Dr. Kaper is the third faculty member of the Department of Microbiology & Immunology to recieve a MERIT award, the others being Drs. Abdu Azad and Stefanie Vogel.

Franscesca Okoye (Farber Lab) has been awarded a pre-doctoral grant from NIH/NIAID (F31 grant). She has also received a travel award to attend a Minority Training Research Forum organized by Temple University in Aventura, FL.

SPOTLIGHT ON: DR. EILEEN BARRY

Dr. Barry grew up in southern New Jersey and received her undergraduate degree at the University of Delaware. She earned her PhD at the Medical College of Virginia in Richmond, working on *Bordetella pertussis* with Alison Weiss. Beginning in 1992, Dr. Barry was a post-doctoral fellow here at UMB in the lab of Glen Morris, where she worked on enterics. In 1996, she was appointed Assistant Professor of Medicine and joined the faculty of the Center for Vaccine Development. As of this semester, she is a secondary appointee to the Department of Microbiology and Immunology.

In her lab, which recently relocated to the Health Sciences Facility II building, Dr. Barry focuses on the development of vaccines for young children in developing countries, for travelers to these countries, for military personnel, and for biodefense purposes. She has developed attenuated strains of *Salmonella typhi* and *Shigella* for use as vaccines. She also works on live vectors for the expression of antigens from other pathogens. Ongoing projects include the development of a multivalent vaccine for *Salmonella pneumococcus* and *Streptococcus pneumoniae* and on a combined *Shigella*/ETEC vaccine.

Dr. Barry is enthusiastic about her work at the CVD because of the unique opportunities provided by the research environment. She explains that her research can progress from basic benchwork to the development of potential vaccines, to preclinical trials, and ultimately, to the passing on of these vaccines to other researchers for possible clinical trials. She currently has two post-doctoral fellows and two research assistants in her lab.

Dr. Barry lectures in the Bacterial Pathogenesis course in our department. She also lectures for the Pathogenic Microbiology course in the Department of Medical and Research Technology (DMRT) and for the bacteriology lab course for medical students. She has had rotation students from the DMRT working in the lab and welcomes rotating students from our department.

PUBLICATIONS

Publications having department students as authors/co-authors are designated with a ♦. Bold face is used to identify department members.

Bordon, J., Evans, P.S., Propp, N., Davis, C.E. Jr, **Redfield, R.R., Pauza, C.D.** Association between longer duration of HIV-suppressive therapy and partial recovery of the V gamma 2 T cell receptor repertoire. J Infect Dis. 2004. Apr 15: 189(8):1482-6.

♦ **Carbonetti, N.H.**, Artamonova, G., **Andreasen, C.**, Dudley, E., Mays, R.M., and Worthington, Z.E.V. Suppression of serum antibody responses by pertussis toxin after respiratory tract colonization by *Bordetella pertussis* and identification of an immunodominant lipoprotein. Infection & Immunity. 2004. 72:3350-3358.

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Fux, C.A., **Shirtliff, M.E.**, Stoodley, P., and Costerton, J.W. Can laboratory strains mirror "real world" pathogenesis? Trends Micro., in press.

♦ Guo, H.G., Pati, S., **Sadowska, M.**, Charurat, M., **Reitz, M**. Tumorigenesis by human herpesvirus 8 vGPCR is accelerated by human immunodeficiency virus type 1 Tat. J Virol. 2004. 78:9336-42.

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levels of apoptogenic and antiapoptotic proteins. FEMS Microbiol. Letters. 2004. 234: 333-341.

Livak, F. In vitro and in vivo studies on the generation of the primary T-cell receptor repertoire. Immunol. Rev. 2004. 200:23-35.

♦ **O'Connell, C.B.**, Creasey, E.A., Knutton, S., Elliott, S., Crowther, L.J., Luo, W., Albert, M.J., **Kaper, J.B.**, Frankel, G., **Donnenberg, M.S**. SepL, a protein required for enteropathogenic *Escherichia coli* type III translocation, interacts with secretion component SepD. Mol Microbiol 2004. 52:1613-1625.

Olaru, A., Patterson, D., Cai, H., and **Livak, F**. Recombination signal sequence variations and the mechanism of patterned T-cell receptor-b locus rearrangement. Mol. Immunol. 2004. 40:1189-1201.

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Rydkina, E., Sahni, S.K., Santucci, L.A., Turpin, L.C., Baggs, R.B., and **Silverman, D.J**. Selective modulation of antioxidant enzyme activities in host tissues during *Rickettsia conorii* infection. Microb. Pathogen. 2004. 36: 293-301.

Shen, S., Mascarenhas, M., Rahn, K., **Kaper, J.B.**, and Karmali, M. (2004). Evidence for a hybrid genomic island in verocytotoxin-producing *Escherichia coli* CL3 (serotype O113:H21) containing segments of EDL 933 O islands 122 and 48. Infection and Immunity. 2004. 72:1496-1503.

Shirtliff, M.E., Leid, J.G., and Stewart, P. Novel strategies to prevent or eliminate biofilms. Microbes Infect., in press.

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♦ **Snyder, J.A.**, Haugen, B.J., Buckles, E.L., Lockatell, C.V., Johnson, D.E., **Donnenberg, M.S.**, Welch, R.A., Mobley, H.L.T. The transcriptome of uropathogenic *Escherichia coli* during urinary tract infection. Infection and Immunity., in press.

Stokes, N.R., Zhou, X., Meltzer, S.J., and **Kaper, J.B**. Transcriptional responses of intestinal epithelial cells to infection with *Vibrio cholerae*. Infection and Immunity. 2004. 72:4240-4248.

Tabrizifard, S., Olaru, A., Plotkin, J., Fallahi-Sichani, M., **Livak, F.,** and Petrie, H.M. Analysis of transcription factor expression during discrete stages of post-natal thymocyte differentiation. J. Immunol. 2004. 173:1094-1102.

♦ Torres, A.G., **Kanack, K.J.**, Tutt, C.B., Popov, V., and **Kaper, J.B**. Initial characterization of the second long polar fimbriae of *Escherichia coli* O157:H7. FEMS Microbiology Letters, in press.

Ward, I.M., Reina-San-Martin, B., Olaru, A., Minn, K., Tamada, K., Lau, J.S., Cascalho, M., Chen, L., Nussenzweig, A., **Livak**, F., Nussenzweig M.C., and Chen, J. 53BP1 is required for class switch recombination. J. Cell Biol. 2004. 165:459-464.

Zhou, X., Gao, D.Q., Michalski, J., Benitez, J.A., and **Kaper, J.B**.. Induction of interleukin-8 in T84 cells by *Vibrio cholerae*. Infection and Immunity. 2004. 72:389-397.

MEETINGS AND POSTERS

Kristen Kanack (Kaper lab), **Rebecca Brady** (Shirtliff lab), **Sandy Jacobsen** (Mobley lab), and **Jennifer Snyder** (Mobley lab) all presented posters at the 104th General Meeting for Microbiology in New Orleans, LA in May.

Jennifer Snyder was an invited speaker at The University of Washington Symposia on Urinary Tract Infections on June 15 in Seattle.

Dr. Martin Flajnik gave talks at NIH (May), the FASEB Meeting on "Lymphocytes and Antibodies" in Saxtons River, VT (June), and the University of Mississippi (July). Dr. Flajnik also organized symposia at the AAI Meeting in Washington, DC (April) and at the FASEB Meeting listed above.

Dr. Ferenc Livak presented at the FASEB/American Association of Immunologists Annual Conference in June in Saxtons River, VT.

Dr. Mark Shirtliff presented at the Management of Battlefield Tissue Injury, Toronto, Canada on June 28. Dr. Shirtliff also presented at the 104th General Meeting of the American Association for Microbiology, New Orleans, LA from May 23-27.

Drs. Carbonetti, Kaper, Nataro, and Donnenberg attended the Gordon Conference on Microbial Toxins and Pathogenesis in Andover, NH in July.

Vaishali Mane (Farber lab) presented at two meetings this spring/summer: Experimental Biology Conference in Washington, DC (April) and the 12th International Congress of Immunology and 4th Annual Conference of FOCIS in Montreal, Canada (July).

RECENT GRADUATES

Rhoel Dinglasan, Spring 2004. Rhoel was born in Manila, Philippines and grew up in Hong Kong. He received his Bachelors degree in 1994 from the University of Virginia in Charlottesville, VA. He then became a senior laboratory technician at the Beirne Carter Center for Immunology at UVA Health Science Center where he worked from 1994-1996. Rhoel received his Masters degree in Public Health in 1998 and his Masters degree in Philosophy/Science from Yale University. He started his PhD work at Yale in 1998 and transferred to UMB to Dr. Azad's lab in 2002 after his former PI left for Australia. Rhoel just received a post-doctoral position at Johns Hopkins School of Public Health where he will work with Dr. Marcelo Jacobs-Lorena. His future plans are in academia. His hobbies are the arts, scuba diving, and global backpacking/trekking. His family still lives in the Philippines and has no plans to move to the US. Rhoel and his wife are hoping to get two Yorkies (Urchin and Mr. Pimms), one pug (Rocco), and two bulldogs (Wilson and Petunia) very, very soon.

Jean Lim, Spring 2004. Jean was born outside of Philadelphia and raised primarily by her grandmother in Baltimore. She did her undergraduate work at Loyola College in Maryland and worked at the Johns Hopkins Univirsity Immunogenetics Laboratory while in school. She started graduate school in 1996 and chose Tony Devico's lab in 1999. Her dissertation work was to on the HIV suppressor molecule RANTES and the regulation of its activity through post-translational processing by proteases. She is planning on staying in the field of chemokines and disease and is currently interviewing for post-doctoral positions. Jean says that her grandmother played a large role in her graduate career by calling her every morning to wake her up. She never forgot and was always on time. Her hobbies include cooking, tennis, painting, piano, watching movies, and traveling, all of which she will resume now that she has graduated!

Laura Quinn Leverton defended her thesis entitled "An Examination of Enteropathogenic *Escherichia coli* Virulence Gene Regulation" on April 23rd.

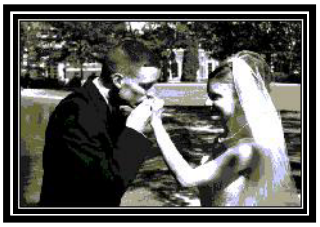
Laurel Burall defended her thesis entitled "Identification of Novel *Proteus mirabilis* Virulence Factors by Signature-Tagged Mutagenesis" on May 5th.

CONGRATULATIONS!!!

June Green's son, Jason Molidor, graduated from the University of Maryland Law School on May 21 and began working for US Airways Legal Counsel in Crystal City, VA on June 1st. Her daughter, Jenna Molidor, graduated from Joppatowne High School and will attend Salisbury University.

Riham El-Asady (Hadley lab) defended her dissertation on August 17. She will be graduating this fall.

Andrew Hebbeler (Pauza lab) completed the Columbia Triathlon, his first Olympic distance triathlon, held in Columbia, MD on May 23rd.



Rebecca Brady (Shirtliff lab) and Sean Brady were married on July 24, 2004 in Elkland, PA. The couple honeymooned in Ocho Rios, Jamaica and resides in Beltsville, MD.

Dr. Martin Flajnik entertained everybody at the Crab Feast on August 26, 2004, by singing karaoke. He performed "New York, New York" and surprised us with his talent!

Dr. Natalia Cuesta (Vogel lab) made her debut as a flamenco dancer with Arte Flamenco dance company at the Kennedy Center Millennium Stage on May 21, 2004.

Dr. Rhoel Dinglasan (Azad lab) married Patricia Michelle Strickler on May 1st. Patricia is finishing up her Ph.D. in Microbiology at Yale University.

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We welcome your comments and suggestions.

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