



EXCITING PROGRAM SCHEDULED FOR IES CONFERENCE

Robert Munford, IES President

The 7th Biennial Conference of the International Endotoxin Society will be held in Arlington, VA (Washington, DC), on July 18-21, 2002. On-line registration, hotel reservation, and meeting information can be found at <http://www.kumc.edu/IES/conference.html>.

The Program Committee has assembled a diverse, timely, and scientifically outstanding program. The following is an incomplete preview:

- In a talk in the opening session, Charles Esmon, who discovered protein C, will discuss “Sepsis as the interface between the inflammatory and coagulation cascades.”
- **LPS recognition and signaling.** Jules Hoffman (Strasbourg) will open the meeting with a talk on “Lessons from the Toll pathway from *Drosophila*.” In another session, Doug Golenbock (Worcester, MA) will present an overview of agonist recognition by Toll-like receptors and the key downstream signaling pathways. Others scheduled to speak on LPS recognition or signaling include Luke O’Neill (Dublin), Stefanie Vogel (Baltimore), Masahiro Nishijima (Tokyo), Kensuke Miyake (Saga), and David Underhill (Seattle). In another session, Rolf Schumann (Berlin) will discuss the role of TLR polymorphisms in susceptibility and response to disease.
- **Genomics.** Clare Fraser (TIGR, Rockville) will discuss “What we can learn from deciphering genomes of microbial pathogens,” and David Relman (Palo Alto) will talk on “Studying microbial-host interactions using microarrays.” Functional genomics as it applies to the study of human responses to LPS will be addressed by Anthony Suffredini (Bethesda), and Eric Hoffman (Bethesda) will use his lab’s results from another field, muscle development, to illustrate the power of the genomics approach. Bruce Beutler (La Jolla) will describe a new approach to identifying genes that influence host responses to LPS and other microbial agonists.
- In the areas of **LPS chemistry and microbiology**, Chris Raetz (Durham) will discuss the lipid A “flippase” in the Gram-negative bacterial outer membrane, the genetics and biochemistry of O-antigen assembly will be presented by Chris Whitfield (Guelph), and Peter van der Ley (Bilthoven) will speak on “Lessons learned from an LPS-deficient meningococcus.” The *phoP/Q* system and lipid A adaptations will be discussed by Robert Ernst (Seattle). Shoichi Kusumoto (Osaka) will provide an update on the chemical synthesis of lipid A.

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INSIDE:

E-Version of Newsletter Offered

IES members may now choose to receive the *Endotoxin Newsletter* electronically instead of by conventional mail, and in the process can help to reduce the cost to the Society of distributing the newsletter as well as impact on the environment. Members electing to receive the newsletter in this way will be notified whenever a new edition of the newsletter has been posted to the IES website (<https://www.kumc.edu/IES/news.htm>). Members can then go to the website and download a pdf version of the newsletter. The newsletter is typically available on the website about one week prior to its arrival by conventional mail, and it is available on

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IES WEBSITE
www.kumc.edu/IES/

Editor's Note: We all know how difficult it is to keep up with the many diverse areas of endotoxin science, and **Recent Progress in Endotoxin Research** is intended to help our readers in this quest. In this section, recent papers that have caught the attention of IES Council members are described. We hope to make this section a regular feature of the Newsletter, and contributions from readers are encouraged. We ask, however, that you please refrain from submitting research conducted in your own group. Please contact the Editor, at the address shown on the masthead, if you wish to submit information for this section.

RECENT PROGRESS IN ENDOTOXIN RESEARCH - #3

CHEMISTRY: One LPS structural element that is implicated in resistance of bacteria to host cationic peptides is the **addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) to the phosphate groups of lipid A**. In *Salmonella*, these regulated modifications are activated by conditions expected to exist within the phagolysosome. In a series of 3 recent papers, Raetz and colleagues have provided insight into the biochemistry of the L-Ara4N residues. They described the formation of the activated precursor UDP-L-Ara4N,¹ the structure of a novel undecaprenol monophosphoryl- α -L-Ara4N intermediate² and a glycosyltransferase that uses this intermediate as a donor of L-Ara4N for lipid A modification.³ The location of these modifications in the periplasm raises interesting questions concerning export of the L-Ara4N donor.

MICROBIOLOGY: Steeghs *et al.*⁴ reported the properties of a fascinating and unprecedented **mutant of *Neisseria meningitidis* that is viable despite the absence of lipid A**. The normally lethal effect of the *lpxA* (UDP-GlcNAc acyltransferase) mutation is tolerated in *N. meningitidis* provided that a capsular polysialic acid polymer is produced. It remains to be established whether the lipid anchor of this group 2 capsule functionally replaces lipid A. The work raises interesting questions concerning outer membrane structure and function in this and other bacteria.

MICROBIAL GENOMICS: In a **genomic-based analysis of lipooligosaccharide variants in *Campylobacter jejuni***, Gilbert *et al.*⁵ reported the structure and function of the LPS biosynthesis loci from 11 isolates expressing a total of 8 different ganglioside mimics in their LPS. Several different genetic mechanisms were identified in the generation of LPS diversity. These include

the presence of different structural genes, phase-variable gene expression, mutations that lead to inactivation of specific glycosyltransferases, and mutations that result in glycosyltransferases with altered specificity.

PATHOLOGY: C.W. Ang and others recently found that the expression of ganglioside mimics by *Campylobacter jejuni* isolates is a risk factor for the development of **post-Campylobacter neuropathy** (Guillain-Barré and Miller Fisher syndromes).⁶ They add to the evidence that exposure to *Campylobacter* LPS determines the anti-ganglioside antibody specificity and clinical features of patients with these syndromes.

Does endotoxin, delivered in a Trojan Horse, cause River Blindness? *Onchocercus volvulus* is the filarial nematode that causes River Blindness, a chronic disease that afflicts hundreds of thousands of persons in Africa. Blindness occurs when microfilariae migrate to the eye and die, eliciting a vigorous host inflammatory response to the degenerating worms. Only recently have scientists realized that *Onchocercus volvulus* microfilariae are infected by *Wolbachia* bacteria. Evidence is mounting that this inflammatory response is elicited by *Wolbachia* endotoxin (and perhaps other bacterial molecules) in a Toll-like Receptor 4-dependent process.⁷⁻⁹ *Wolbachia* bacteria also live symbiotically within *Wuchereria bancrofti*, the etiological agent of lymphatic filariasis, and thus may contribute to the pathogenesis of this disease as well.

IMMUNOLOGY: In *Drosophila*, the response to various microorganisms involves different recognition and signaling pathways, as well as distinct antimicrobial effectors. Rutschmann *et al.* recently reported that the *Toll* pathway is required for resistance to some Gram-positive bacterial infections and that the *immune deficiency (imd)* pathway is not.¹⁰ This differs from the pattern in vertebrates, where TLR4 mediates the activation of NF- κ B

by LPS, whereas TLR2 plays a similar role in response to peptidoglycan. In *Drosophila*, the same Toll receptor is required for the response to both fungal and Gram-positive bacterial elicitors, suggesting that in this system the recognition step that discriminates between these distinct pathogens takes place upstream of the Toll receptor. Indeed, Michel and colleagues recently reported that the **peptidoglycan recognition protein SA (PGRP-SA)** plays a major role in the activation of the *Toll* pathway.¹¹ Orthologs of such "upstream of TLR" microbial recognition molecules have not been identified in vertebrates.

The first **mechanistic basis for differential patterns of gene expression** activated by TLR4 and TLR2 agonists has been described by Toshchakov and others.¹² They found that the ability of LPS (TLR4), but not peptidoglycan (TLR2) to activate the STAT pathway in murine macrophages resulted from the activation of IFN- β by LPS. TLR4-induced IFN- β was MyD88-independent but TIRAP-dependent.

Several recent studies have found important roles for the central nervous system in controlling systemic reactions to LPS. Perhaps the most intriguing of these has been the demonstration, by Kevin Tracey's group, of a **"cholinergic anti-inflammatory pathway"** in which stimulation of the vagus nerve inhibits TNF production by the liver and other organs. These workers recently reported that CNI-1493, a tetravalent guanlylhydrazone molecule that inhibits systemic inflammation, acts by stimulating the vagus nerve, even when the drug is administered outside the CNS.¹³ Moreover, stimulation of either the right or left vagus attenuated serum and myocardial TNF, but not pulmonary TNF, in response to endotoxin challenge. Does the inability of the cholinergic anti-inflammatory pathway to diminish pulmonary TNF production make the lung more susceptible to injury (ARDS) during sepsis?

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Reader's Question:

How Do You Get Rid of Contaminating Endotoxin?

**Kent Myers,
Endotoxin Editor**

I received a question recently from a reader who had been trying unsuccessfully to remove a stubborn endotoxin contamination from a protein preparation. The reader had tried filtering the protein through a variety of ion exchange membranes and had also tried precipitation, all without success. Denaturation was apparently not an important concern with this protein. I suggested that the reader try dissociating the endotoxin from the protein with detergents prior to purification. For example, a dialyzable non-ionic detergent might be used to enhance the interaction of the endotoxin with the charged groups on an ion-

exchange membrane. Or, detergents could be combined with phenol extraction in a manner analogous to the method developed by Stephanie Vogel et al. to solve the reverse problem of removing contaminating proteins from endotoxin (*J Immunol* **165**, 618-22[2000]). I am sure that many of the readers of this newsletter have encountered this problem and have experiences and/or insights that would be of interest to the general membership. Please pass along any such comments to me at the address shown on the masthead, and I will see that they are included in a future issue of the newsletter. □

E-Version of Newsletter

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the website to anyone. Nancy Pollman will be contacting all members for whom we have valid email addresses within the next few weeks to ask if they wish to continue receiving hard copies of the newsletter, or if they would prefer to only receive the notification by email. The default method of distributing the newsletter will continue to be by conventional mail. Anyone wishing to contact Nancy regarding this new option may do so at npollman20@aol.com. □

Recent Progress in Endotoxin Research

(Continued from previous page)

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Election of Officers to be Held

The Nomination Committee has submitted the slate of candidates for the elected positions in the IES. A ballot is enclosed with this newsletter, and all active members are strongly encouraged to vote. An active member is defined as anyone who has paid their dues up through 2001. Ballots may be returned either by fax or mail using the directions on the ballot. Any questions regarding the elections should be directed to Dick Silverstein, IES Secretary, at either +1-913-588-6954 or rsilvers@kumc.edu. □

Nominations Sought For Honorary Lifetime Members

Nominations are now being solicited for individuals to be awarded Honorary Lifetime Membership in the IES. According to the IES Constitution, one individual may be designated each year as an Honorary Life Member of the Society, in recognition of his or her career contributions to the knowledge and understanding of bacterial endotoxins. Nominees for the award are reviewed by the Governing Council prior to each biennial conference of the IES, and two individuals (for each year since the last conference) are selected for approval by the general membership. Election to Honorary Life Member status requires a two-thirds majority vote of the membership attending the biennial business meeting. Honorary Life Members have all the rights and privileges of active members but are exempt from Society dues and meeting registration fees. The following individuals have received this honor: Louis Chedid, Sheldon E. Greisman, Arthur G. Johnson, Otto Lüderitz, William R. McCabe, W.T.J. Morgan, Masagasu Nakano, Mary Jane Osborn, Johanna

Schlosshardt, Tetsuo Shiba, Anna-Marie Staub, Lewis Thomas, D.W. Watson, and Otto Westphal. Please send nominations, in the form of a letter or email describing the nominee's history of achievements in endotoxin research, to:

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Exciting Program Schedule

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• A session on **non-TLR signaling** will include talks on NOD and its relationship to inflammatory bowel disease (Daniel Podolsky, Boston), the role of adenosine receptors in responses to LPS (Paul Bertics, Madison), the role of potassium ions in LPS responses (Ulrich Seydel, Borstel), the role of nitric oxide in LPS signaling (Takashi Yokochi, Aichi), and a non-CD14 receptor complex for LPS (Kathy Triantafilou, Portsmouth). Yang (Manhasset) will present an update on "HMG-1 as a cytokine," and David Mosser (College Park, MD) will speak on the ability of Fc receptor signaling to modulate macrophage responses to LPS. Jeff Hasday (Baltimore) will discuss the role of heat shock responses in LPS signaling. IκB-zeta, a new anti-inflammatory molecule that regulates NF-κB, will be described by Tatsushi Muta (Fukuoka).

• **Neuroimmune mediators and host defense.** Considerable evidence points to a critical role for neuroendocrine mediators in host responses to infection. George Chrousos (Bethesda), whose group has pioneered in the study of the hypothalamic-pituitary axis in health and disease, will give an overview of the topic. Kevin Tracey (Manhasset) will discuss his lab's data on the "cholinergic anti-inflammatory pathway." Charles Dinarello (Denver) will discuss some fresh insights on an old topic, the role of endotoxin in the pathogenesis of fever.

A session on **LPS tolerance and sensitivity** will include an overview of the state of the field by Jean-Marc Cavaillon (Paris), as well as talks by Charles McCall (Wake Forest) on the role of PI-3 kinase in

LPS tolerance, Marina Freudenberg (Freiburg) on the role of interferon beta in the pathogenesis of sepsis, and Klaus Heeg (Munich) on synergism between LPS and superantigens.

Poster sessions are scheduled, and meeting attendees are encouraged to submit abstracts for poster presentations. Note that the deadline for abstract submission has been extended to April 25. In addition to the talks described above, **several abstracts will be selected for oral presentations** and space in the program is being held for important "late breaking" developments.

Early registration is strongly encouraged, before the meeting and hotel fees increase in July. □

Join us for an exciting meeting!

It's Easy to be a Tourist in Washington DC

Kent Myers, Endotoxin Editor

Attendees at the IES Biennial Conference in Washington DC will have plenty of things to do during the times when they are not hearing about the latest endotoxin research findings. This beautiful city offers a superb public transportation system, a diverse selection of outstanding museums, and spectacular sightseeing opportunities, and it is a truly great city to visit as a tourist. My favorite tourist destination in Washington DC is the immense central area known as The Mall. As can be seen on the useful map at <http://sc94.ameslab.gov/TOUR/tour.html>, this area contains an almost overwhelming selection of museums, monuments, and significant government institutions. Meeting participants will be able to travel to The Mall quickly and easily via the Metro, by taking the Yellow Line from Crystal City to l'Enfant Plaza. (Go to www.wmata.com/metro/rail/systemmap.htm for a subway map and additional Metro information). I particularly enjoy visiting the National Gallery of Art, the Hirshhorn Museum, and the National Air and Space Museum (a must if you have kids!). It is also wonderful to just stroll around the beautiful grounds and reflect on such monuments as the Vietnam Veteran's Memorial and the Lincoln memorial. There are a large number of other attractions that are within easy walking distance of The Mall, including the National Portrait Gallery, Ford's Theater (still functional), and The White House. I strongly recommend that you put this area at the top of your list of places to visit while you are at the meeting. □

Workshop: Genomics for Beginners (How to Collect and Analyze Data from Microarrays)

Thursday, July 18, 2002

Crystal Gateway Marriott Hotel, Arlington, VA
(in the AM...exact time and room to be announced)

Presenter: Eric Hoffman, M.D.
Director and Professor
Research Center for Genetic Medicine
Children's National Medical Center
Washington D.C.

Interested in attending? Register for IES 2002, then email Robert.Munford@utsouthwestern.edu for further information.

A workshop titled **What Is Endotoxin?**, on the basics of working with endotoxin/LPS and lipid A, is still being considered. Please contact Robert Munford at the email address shown above if you would be interested in attending such a workshop.

SOCIETY NEWS

New IES Members

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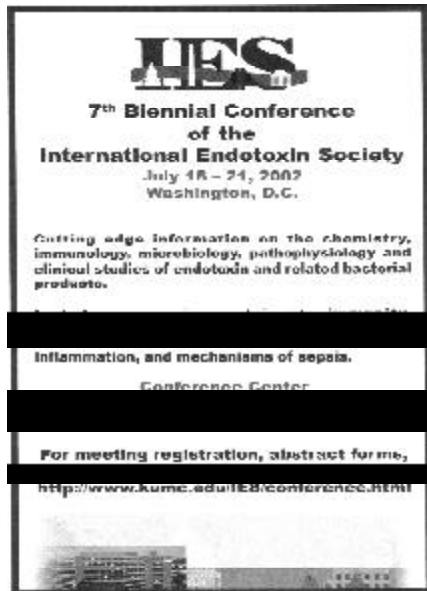
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**Also, welcome back
to all past members
that have re-joined!**

Save the date! July 18-July 21, 2002
7th Conference of the International
Endotoxin Society
Bethesda, Maryland USA



IES Contact Information

Need to update your address information? Wondering why you have not received a copy of the newsletter? (It probably has not been mailed yet!) Want to pay your dues but are not sure how? You can get answers to these and all other questions related to your IES membership from the following individuals. See your directory for other means of contact, i.e. mailing addresses and phone numbers.

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The deadline for abstract submission has been extended to April 25

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