

# ENDOTOXIN NEWSLETTER

## IES NEWS UPDATES



Here are some updates on topics of current interest to the IES membership:

- The 7th Conference of the IES will be held in Washington, D.C., on Thursday, July 18, 2002, through Sunday, July 21, 2002. The meeting venue has been finalized and is at the Natcher auditorium, located on the Bethesda campus of the National Institutes of Health. The Natcher center is a modern facility with ample room for large and small sessions. It is located next to the National Library of Medicine on the wooded NIH campus and is very close to a Metro stop. The NIH is a short subway ride from the center of Washington, D.C., with its outstanding museums and public buildings. Current information on the conference will be posted at [www.kumc.edu/IES/conference.html](http://www.kumc.edu/IES/conference.html) as it becomes available. Questions regarding the conference can also be directed to Anthony Suffredini ([asuffredini@nih.gov](mailto:asuffredini@nih.gov)), chairperson of the Local Host Committee;
- The IES website ([www.kumc.edu/IES](http://www.kumc.edu/IES)) has been updated. The site offers a full range of services, including detailed information on the Society, names and contact information for current IES officers and committee members, access to back issues of the IES Newsletter, subscription and author information for the *Journal of Endotoxin Research*, application and renewal forms (including the option of online dues and *JER* subscription payments), current information on the IES Conference, and more;
- Thanks to the hard work of Dick Silverstein, Nancy Pollman, and others, the new IES Membership Directory was mailed recently to all IES members in good standing. This directory is an important benefit of membership in the IES, and includes complete contact information for all members in the Society as well as useful information about the Society itself, such as the Constitution and By-Laws, lists of current officers and committee members, IES award winners, Honorary Life Members, and past officers, and a breakdown of membership by country. Please contact Dick Silverstein ([rsilvers@kumc.edu](mailto:rsilvers@kumc.edu)) if you did not receive your copy;
- Work on the on-line directory is also moving forward, and a beta version has been prepared and distributed to IES officers for evaluation. Comments on the directory will be received during the upcoming meeting of the Governing Council (see below);
- The next meeting of the IES Council will be in Chicago, Illinois, on Friday, September 21, 2001, immediately prior to the ASM ICAAC meeting;
- We are continuing to compile a list of companies that manufacture endotoxin-related products, with the intention of making this list available on the IES website. This topic will be discussed at the upcoming meeting of the Governing Council. Please contact Bob Munford ([Robert.Munford@UTSouthwestern.edu](mailto:Robert.Munford@UTSouthwestern.edu)) if you would like further information on this proposed service. □

[www.kumc.edu/IES/](http://www.kumc.edu/IES/)

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## JER Honors Ernst Th. Rietschel

Prof. Dr. Ernst Th. Rietschel received an unusual but heart-felt birthday card when the current issue of the *Journal of Endotoxin Research* (volume 7, issue 2) arrived in his mailbox. This issue was published as a *Festschrift* (or commemorative publication) in honor of Dr. Rietschel on the occasion of his 60th birthday, and it comprises an impressive collection of peer-reviewed papers contributed by his many friends and colleagues. (The Table of Contents for this issue can be found on p.3.) Jack Levin, Editor-in-Chief of *JER*, explained in the accompanying editorial that it was a "pleasant and rewarding task" to assemble this

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

Editor's Note: It's often difficult to keep up with the many diverse areas of endotoxin science. Recent Progress in Endotoxin Research is a new section in which a potpourri of papers that caught the attention of IES Council members recently are briefly described. We hope to make this section a regular feature of the Newsletter, and members who wish to recommend articles for upcoming issues are encouraged to contact Kent Myers, Editor, at the address shown on p.1.

# RECENT PROGRESS IN ENDOTOXIN RESEARCH

**CHEMISTRY:** *Aquifex pyrophilus* is a phylogenetically ancient member of the *Bacteria*. It grows at temperatures up to 95°C and is a close relative of *Aquifex aolicus*, whose genome sequence has been reported. While it was not known whether *A. pyrophilus* contained LPS, the genome of *A. pyrophilus* contains orthologs of a number of genes that encode LPS biosynthesis enzymes in the Enterobacteriaceae. *A. pyrophilus* produces smooth LPS and the novel structure of the lipid A portion has been elucidated by Plötz *et al.*<sup>1</sup> It consists of a  $\beta$ -1'→6-linked 2,3-diamino-2,3-dideoxy-D-glucopyranose disaccharide, modified at the 1 and 4' positions with D-galacturonic acid residues. The native lipid A is pentaacylated.

Kusumoto's group<sup>2</sup> has reported the first total synthesis of a complete deep rough mutant *Escherichia coli* type LPS, surely a task that required outstanding experimental skills. The availability of a synthetic LPS is very important for at least two reasons: one is its clearly defined chemical structure, which should allow the determination of detailed structure-activity relationships. The second reason concerns the absence of possible contamination, in particular with proteins and lipoproteins, which have been found to occur very frequently and which can hardly be detected below 1 or 2%.

**IMMUNOLOGY:** Werts *et al.*<sup>3</sup> reported that *Leptospira interrogans* contains an unusual LPS that signals macrophages via TLR2. This is very reminiscent of the recent report by Hirschfeld *et al.*<sup>4</sup> that *P. gingivalis* LPS signals through TLR2 and leads to differential gene expression when compared with TLR4-mediated signaling by *E. coli* LPS. Both studies extend the discussion as to which cellular receptor on macrophages mediates the LPS signal from different microorganisms. Although the structure of the *Leptospira* "LPS" is not known, an accompanying editorial by

Golenbock and Fenton<sup>5</sup> notes that the *Leptospira* genome has several orthologs of lipid A biosynthetic genes.

A non-TLR intracellular signaling pathway for LPS has been proposed by Inohara *et al.*<sup>6</sup> Nod1 is a member of a family of intracellular proteins with structural homology to apoptosis regulators Apaf1/Ced-4. Expression of Nod1 in 293T cells allowed LPS-specific, yet TLR4- and MyD88-independent, activation of NF- $\kappa$ B. The authors suggest that Nod1 and Nod2 are mammalian counterparts of plant disease-resistance gene products that may function as cytosolic receptors for LPS.

**MICROBIOLOGY:** While knowledge of the biosynthesis of LPS has developed significantly in the last decade, crucial unanswered questions surround the mechanism(s) by which LPS is transferred from its site of synthesis in the cytoplasmic membrane to its final location in the outer leaflet of the outer membrane. *MsbA* is an essential ABC exporter previously implicated in the export of newly synthesized LPS across the cytoplasmic membrane of *E. coli*. Using a temperature-sensitive *msbA* point mutant, the Raetz laboratory has now determined that an *msbA* defect influences phospholipid transfer as well as LPS assembly. *MsbA* therefore plays an essential role in general lipid trafficking in *E. coli*.<sup>7</sup>

**PATHOLOGY:** The two faces of interleukin-10 (IL-10) have been exposed by Sewnath *et al.*<sup>8</sup> Using a mouse model, they found evidence that endogenous IL-10 facilitates the growth and dissemination of bacteria during *E. coli* peritonitis yet it also protects mice from dying by attenuating the systemic response by a mechanism that involves inhibition of TNF release.

Leukocytes from septic patients exhibit repressed production of inflammatory cytokines, such as IL-1 $\beta$ , and elevated production of anti-inflammatory ones, such as IL-1Ra. Learn *et al.*<sup>9</sup> found that the selective production of IL-1Ra is

caused by efficient translation of IL-1Ra messenger RNA, which in turn is driven by a phosphatidylinositol-3-kinase-dependent pathway. This LPS-responsive signaling pathway is maintained despite repression of cytokine gene transcription via the TLR-IRAK pathway. The findings shed new light on the phenomenon of endotoxin tolerance. □

## References:

1. Plötz BM, Lindner B, Stetter KO, Holst O. Characterization of a novel lipid A containing D-galacturonic acid that replaces phosphate residues. The structure of the lipid A of the lipopolysaccharide from the hyperthermophilic bacterium *Aquifex pyrophilus*. *Journal of Biological Chemistry* 2001; **275**: 11222-11228.
2. Yoshizaki K, Fukuda N, Sato K *et al.* First total synthesis of Re-type lipopolysaccharide. *Angew Chem Int Ed* 2001; **40**: 1475-1480.
3. Werts C, Tapping RI, Mathison JC *et al.* Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2001; **2**: 346-352.
4. Hirschfeld M, Weis JJ, Toshchakov V *et al.* Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infection and Immunity* 2001; **69**: 1477-1482.
5. Golenbock DT and Fenton MJ. Extolling the diversity of bacterial endotoxins. *Nat Immunol* 2001; **2**: 286-288.
6. Inohara N, Ogura Y, Chen FF, Muto A, Nuñez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *Journal of Biological Chemistry* 2001; **276**: 2551-2554.
7. Doerrler WT, Reedy MC, Raetz CR. An *Escherichia coli* mutant defective in lipid export. *J Biol Chem* 2001; **276**: 11461-11464.
8. Sewnath ME, Olszyna DP, Birjmohun R *et al.* IL-10-deficient mice demonstrate multiple organ failure and increased mortality during *Escherichia coli* peritonitis despite an accelerated bacterial clearance. *Journal of Immunology* 2001; **166**: 6323-6331.
9. Learn CA, Boger MS, Li L, McCall CE. The phosphatidylinositol 3-kinase pathway selectively controls sIL-1RA not interleukin-1 production in septic leukocytes. *Journal of Biological Chemistry* 2001; **276**: 20234-20239.

## Letter to the Editor: Use of Defined Endotoxin Preparations

Dear Editor,

I read with great interest "A Lesson From Paris" that appeared in the *Endotoxin Newsletter*, Volume 11, Number 1, Spring 2001. I regret that I could not attend the meeting, which, like previous meetings, dealt with important points regarding the research on endotoxin.

Already in 1989 I had the need to use defined concentrations of the best preparations of endotoxin then available when I designed the protocol of a project to study the endotoxemia sequelae in rhesus monkeys as an experimental model of infection in humans. On one hand, I had to meet the strict requirements of the Guide for the Care and Use of Laboratory Animals, as promulgated by the National Resource Council; on the other hand, information on the doses of endotoxin that could kill monkeys was not available. In addition, I needed to use defined endotoxin concentrations to study the correlation between responses, doses and plasma concentrations. I had a Senior Research Associateship from the National Research Council to do, in two years, the project. I believe that it had not been feasible to use lipid A preparations in the project.

To circumvent those difficulties, and with the information that monkeys were much more resistant to endotoxin than were humans or apes, I did a pilot experiment using the FDA endotoxin standard, Lot EC-5, at a concentration much higher than those of this endotoxin previously reported in experiments with humans and apes.

Because I confirmed that, under the experimental conditions used, rhesus monkeys were much more resistant to endotoxin than humans – therefore, very high concentrations would be needed to mimic severe clinical situations – and because of the limited availability of that highly purified endotoxin, I selected a commercially available endotoxin similar to that of the FDA on the basis of its specific activity (3 E.U./ng vs. 5 E.U./ng). A defined specific activity was also necessary to study the correlation of doses and plasma concentrations with responses in individual animals as well as to have experimental conditions that could be reproduced easily in other laboratories.

Regarding the first part of the project, I and my collaborators published an article entitled "Differential susceptibility of rhesus monkeys to high doses of endotoxin" in the *Journal of Endotoxin Research* 1995, vol. 2:411-420. As a continuation of the project, we published an article entitled "Rhesus differential susceptibility to endotoxin is not associated with activation of plasma prekallikrein" in *Immunopharmacology* 1999, vol. 43:265-271. Further research – already presented at the 1998 International Meeting of Endotoxin and the 1999 International Meeting of Thrombosis and Haemostasis – is in preparation to be submitted.

Sincerely,  
Dulce Veloso, Ph.D.

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### JER Honors Ernst Th. Rietschel

issue in recognition of the many contributions made by Dr. Rietschel to the study of bacterial endotoxins. As described by Dr. Levin, these contributions range from his incisive studies on the chemical and physical properties of endotoxin, to his outstanding leadership of the Research Institute at Borstel, to his pivotal role in the inception and growth of the International Endotoxin Society.

This issue of *JER* is also notable in that it marks the inauguration of an online access service for the journal. The service, available at [www.ingenta.com](http://www.ingenta.com), allows issues from volume 5 onward to be browsed or searched. Titles and abstracts are available to everyone, and full text versions of articles are accessible to paid subscribers. Take a look at this useful service when you have the chance. □

### Table of Contents of *Journal of Endotoxin Research*, vol. 7, no. 2, "A Festschrift in Honor of Ernst Th. Rietschel."

Immunodepression in sepsis and SIRS assessed by *ex vivo* cytokine production is not a generalized phenomenon: a review  
**Cavaillon J.-M.; Adib-Conquy M.; Cloëz-Tayarani I.; Fitting C.**

Chemical structure and biological activity of a lipid A component from *Helicobacter pylori* strain 206  
**Suda Y.; Kim Y.-M.; Ogawa T.; Yasui N.; Hasegawa Y.; Kashihara W.; Shimoyama T.; Aoyama K.; Nagata K.; Tamura T.; Kusumoto S.**

Cytokine synthesis in the liver of endotoxin-tolerant and normal rats during hemorrhagic shock  
**Ackermann M.; Reuter M.; Flohé S.; Bahrami S.; Redl H.; Schade F.U.**

Structural and serological characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from *Acinetobacter* strain 96 (DNA group 11)  
**Vinogradov E.V.; Pantophlet R.; Brade H.; Holst O.**

A monoclonal antibody with specificity for the genus *Klebsiella* binds to a common epitope located in the core region of *Klebsiella* lipopolysaccharide  
**Brade L.; Podschun R.; Brade H.**

Synthesis and immunochemical characterization of neoglycoproteins containing epitopes of the inner core region of *Pseudomonas aeruginosa* RNA group I lipopolysaccharide  
**Reiter A.; Brade L.; Sanchez-Carballo P.; Brade H.; Kosma P.**

Structural and biological characterisation of a novel tetra-acyl lipid A from *Escherichia coli* F515 lipopolysaccharide acting as endotoxin antagonist in human monocytes  
**Zähringer U.; Salvetzki R.; Wagner F.; Lindner B.; Ulmer A.J.**

Influence of acyl chain fluidity on the lipopolysaccharide-induced activation of complement  
**Wiese A.; Grünwald P.; Schaper K.-J.; Seydel U.**

TNF- $\alpha$  hyper-responses to Gram-negative and Gram-positive bacteria in *Propionibacterium acnes* primed or *Salmonella typhimurium* infected mice  
**Merlin T.; Gumenscheimer M.; Galanos C.; Freudenberg M.A.**

## **New IES Members**

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School of Biomedical & Life Sciences  
Surrey UK

Lynn Stoll, PhD  
University of Iowa  
Iowa City, IA USA

*Welcome*

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

## **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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