A Publication of the International Endotoxin and Innate Immunity Society

ENDOTOXIN NEWSLETTER

January 2021

A Letter from the IEIIS President

Koichi Fukase

Dear Friends and Colleagues,

The COVID-19 pandemic has caused great damage to individuals, the economy, and society. IEIIS has decided to postpone the 16th biennial IEIIS meeting in 2020 to 2021. Although the mortality rate of infected people is decreasing due to improvements in treatment, development of new treatment methods and vaccines remains a high priority. The 16th IEIIS welcomes proposals for related topics and workshops given the importance of the innate immune system in SARS-CoV-2 infection and its complications, especially cytokine storms. Some updates:

2021 MEETING: We are planning an attractive program for the next IEIIS meeting, which will be held October 12-15, 2021, at the Kobe International Conference Center. Kobe, a historical port city in Western Japan, located close to Osaka and Kyoto, is famous for Kobe beef, Japanese sake, and beautiful night views. On the other hand, because it is difficult to predict the COVID-19 situation, we want to hold the meeting as a hybrid-type conference. We are planning an attractive program. Proposed sessions include following topics: mucosal immunity; gut-microbe and immuno-regulation; vaccines and adjuvants; ligands of innate immunity, structure and function; lectins and immuno-regulation; innate immunity and sterile inflammation including lifestyle diseases; shock/sepsis. Keynote lectures will be given by Prof. Shizuo Akira (Osaka University) and Prof. Hiroshi Kiyono (The University of Tokyo). To reaffirm the importance of innate immunity in COVID-19 related challenges and to address it in an international framework, we hope that you will join us to exchange current research discoveries while renewing old friendships and establishing new ones. We look forward to welcoming and meeting you in Kobe or on web.

MEMBERSHIP DRIVE: We are starting a membership drive to increase membership numbers. This will be coordinated with the help of our Membership Chair, Tim Sellati. New is the 2-year membership. There are many advantages with membership ability to join interesting meetings and present your research, you will get a useful network of contacts, you can get advice on career choices and experiments, publish in the *Innate Immunity* journal, and more. This is a good opportunity for you and your friends to become or stay affiliated with the Society!

WEBSITE: At the website you will find information about the Society; ability to join or re-join; contact information for officers, councilors and other people in key positions; information about upcoming events and meetings; awards, and more. If you have any input, please contact Holger Heine at hheine@fz-borstel.de

***InI* JOURNAL:** Our great journal *Innate Immunity* continues to publish high quality reports. New developments are that *InI* has become an open access journal, which will make papers freely available.

If you have any comments or ideas with regard to the Society, please contact me at koichi@chem.sci.osaka-u.ac.jp

Best regards,

Koichi Fukase

Koichi Fukase
IEIIS President 2018-2020

Inside this issue:

IEIIS - INTERNATIONAL ENDOTOXIN
& INNATE IMMUNITY SOCIETY

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IEIIS 2021

16th Biennial Meeting of the International Endotoxin and Innate Immunity Society

Oct 12-15, 2021 - Kobe International Conference Center, Japan

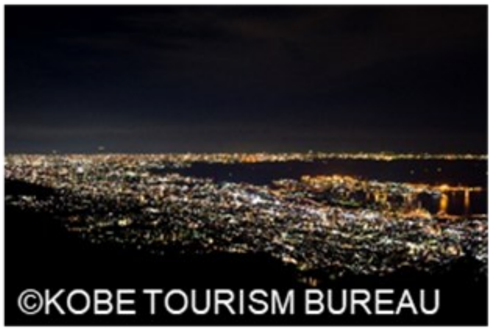
Chair: Koichi Fukase (Osaka University)

It is a great pleasure and honor to host the 16th biennial IEIIS meeting, which will be held October 12-15, 2021, at the Kobe International Conference Center. Kobe, a historical port city in western Japan, located close to Osaka and Kyoto, is famous for Kobe beef, Japanese sake, and beautiful night views.

On the other hand, because it is difficult to predict the COVID-19 situation, we want to hold the meeting as hybrid-type conference. We are planning an attractive program. Proposed sessions include following topics: mucosal immunity; gut-microbe and immuno-regulation; vaccines and adjuvants; ligands of innate immunity, structure and function; lectins and immuno-regulation; innate immunity and sterile inflammation including lifestyle diseases; shock/sepsis.

To reaffirm the importance of innate immunity in COVID-19 related challenges and to address it in an international framework, we hope that you will join us to exchange current research discoveries while renewing old friendships and establishing new ones. We will also provide good opportunities, especially for young scientists and students, to meet and interact with top scientists working in this field and expand their knowledge.

SAVE THE DATES!!



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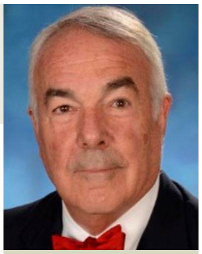
WELCOME TO KOBE, 2021

Check meeting updates on the web sites: https://www.ieiis.org/

IN THE NEWS

Update from Dr. Alan Cross: A Novel Inflammatory Peptide is Now Under Review at the FDA


Alan Cross

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Working with Dr. Raymond Kaempfer at the Hebrew University in Jerusalem, Alan Cross had shown that a CD28 homodimer interface mimetic peptide that blocks the dimerization of CD28 on T cells attenuates the cytokine storm associated with inflammation but does not completely inhibit the immune response. Peptide treatment of mice challenged with superantigens (staphylococcus enterotoxin B or streptococcal exotoxins), live group A streptococci, Gram-negative bacterial LPS, and infection improved outcomes. This peptide (AB103 [Reltecimod]) has progressed through a 290 patient Phase 3 clinical trial where it recently was shown to reduce morbidity in patients with necrotizing soft tissue infections. This peptide is currently under review at the FDA. ▲

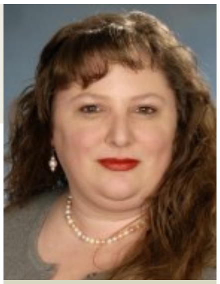
References

Ramachandran G et al. A peptide antagonist of CD28 signaling attenuates toxic shock and necrotizing soft tissue infection induced by *Streptococcus pyogenes*. *J Infect* 2013;**207**:1869.

Ramachandran G et al. CD28 homodimer interface mimetic peptide acts as a preventive and therapeutic agent in models of severe bacterial sepsis and gram-negative bacterial peritonitis. *J Infect* 2015:**211**:995-1003

Bulger EM et al A novel immune modulator for patients with necrotizing soft tissue infections: Results of a multicenter, Phase 3 randomized controlled trial of Relticimod (AB103), *Ann Surgery*; 2020;**272**: 469-478.

Update from Dr. Kari Ann Shirey:
Tips and Tricks for Scientific Figure Making


Kari Ann Shirey

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Over the summer, Dr. Kari Ann Shirey (Assistant Professor, University of Maryland School of Medicine) presented a virtual breakout session at the annual Medical Scientist Training Program (MSTP) retreat for the MD/Ph.D. students at the University of Maryland School of Medicine entitled, "Tips and Tricks for Scientific Figure Making." The breakout session was geared for the first- and second-year graduate students who were just getting started in their laboratory projects and had not been part of writing and figure-making previously. ▲

Dr. Timothy J. Sellati Has Shared the Abstract of a Recent Review He Wrote on Antibody Responses in Lyme Disease


Timothy Sellati

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Although Lyme disease-centric, some of the concepts of how a pathogen can inadvertently or purposefully elicit an adaptive response that is not "up to the challenge" of sterilizing an infected person and conferring life-long protection might be of general interest. *Borrelia burgdorferi* is simply one of several examples of such pathogens. SARS-CoV-2 appears to be one of the more recent examples.

Abstract: Lyme disease (LD), caused by the spirochetal bacterium *Borrelia burgdorferi*, is transmitted by the black-legged tick Ixodes scapularis (Hu, 2016). LD is the fastest growing global tick-borne disease and annually affects >300,000 people in the U.S. alone (Steere et al., 2016). The economic impact is a staggering $1.3 billion dollars per year (Adrion et al., 2015). LD can cause long-term, debilitating symptoms, including arthritis, carditis, and neurological complications (Hu, 2016; Steere et al., 2016). A longstanding question is why antibodies produced during primary infection are not able to completely clear spirochetes or confer protective immunity (Barbour et al., 2008). Antibody titers can remain for years in some LD patients while in others, they wane over time or never develop at all (Kalish et al., 2001). Herein, we describe animal studies that reveal mechanisms behind dysregulated development of adaptive immunity and provide insights that may be relevant to human immunity to *B. burgdorferi* infection.

Sellati, T. J., and D. M. Barberio. (2020) Mechanisms of Dysregulated Antibody Response in Lyme Disease. *Front Cell Infect Microbiol* **10**, 567252. https://doi.org/10.3389/fcimb.2020.567252

Pairing *Bacteroides vulgatus* LPS Structure with Its Immunomodulatory Effects on Human Cellular Models


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The gut microbiota is becoming an increasingly important and active research field due to its vast complexity and its potential medical outcomes. However, the understanding of the molecular interactions between bacterial microbiota and the host immune cells are still not well understood. Gram-negative bacteria are found abundantly within the human microbiota and their presence is of vital importance for the maintenance of the delicate balance between health and disease occurring in the human body. Evidently, the structure and immunological properties of the lipopolysaccharides from the "good" bacteria, i.e. the harmless and/or beneficial components of the Gram-negative microbiota, must be different from those observed for their pathogenic counterparts.

Our team has started a scientific program aimed at exactly define the structure to function relationship of the LPS isolated from the gut microbiota, beginning with our recent publication on the full characterisation of the LPS from *Bacteroides vulgatus* mpk, a prominent commensal bacterium of the human and murine intestine. Through the combined use of chemical, NMR spectroscopic, and mass spectrometric techniques we reported that the LPS from this bacterium has a completely unprecedented and uncommon structure to which corresponds an equally particular immunological activity. The LPS of *B. vulgatus* mpk is, indeed, able to only poorly stimulate the release of pro-inflammatory cytokines while inducing the production of the anti-inflammatory cytokine IL-10 *in vitro* by using human cellular models.

(Continued on page 5)

Pairing *Bacteroides vulgatus* LPS Structure with Its Immunomodulatory Effects on Human Cellular, *continued*

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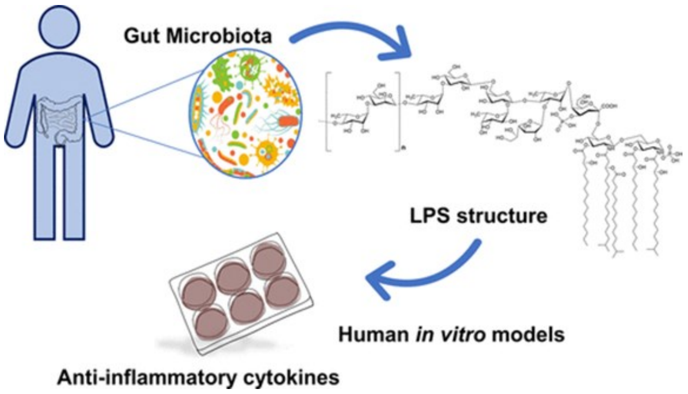
Moreover, this particular LPS was able to activate both the MD2/TLR4- and the TLR2-mediated signalling pathways, i.e. an additional uncommon immunological behaviour compared to other types of LPS. ▲



Di Lorenzo, F., M. D. Pither, M. Martufi et al. (2020) Pairing *Bacteroides vulgatus* LPS Structure with Its Immunomodulatory Effects on Human Cellular Models. *ACS Cent Sci* **6(9)**, 1602–1616. https://doi.org/10.1021/acscentsci.0c00791

Update from Dr. Stephanie Vogel: Important Insights from a Novel Murine Model of Human TLR4 Polymorphisms


Stefanie Vogel

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We have a very exciting paper that was recently accepted by J Experimental Medicine entitled, "A Mouse Model of Human TLR4 D299G/T399I SNPs Reveals Mechanisms of Altered LPS and Pathogen Responses", by:

Katharina Richard, Kurt H. Piepenbrink, Kari Ann Shirey (co-first-authors), Archana Gopalakrishnan, Shreeram Nallar, Daniel J. Prantner, Darren J. Perkins, Wendy Lai, Alexandra Vlk, Vladimir Y. Toshchakov, Chiguang Feng, Rachel Fanaroff, Andrei E. Medvedev, Jorge C. G. Blanco, and Stefanie N. Vogel

Using advanced protein modeling from crystallographic data of human and murine TLR4, we identified homologous amino acid substitutions in mice for the human TLR4 299/399 SNPs which have been associated with LPS-hyporesponsiveness and altered sensitivity to a variety of infectious and non-infectious inflammatory diseases. From there, we used a CRISPR-Cas 9 approach to engineer homozygous "TLR4-SNP" (D298G/N397I) mice on a C57BL/6J background. The responses of these mice and their macrophages were compared to wild-type C57BL/6J and TLR4-/- mice/macrophages for their in vivo and in vitro responses to LPS and to infectious agents previously associated with TLR4. Our data indicate that the TLR4-SNP mice and their macrophages exhibit LPS-hyporesponsiveness, decreased expression of macrophage surface and total TLR4, a reduction in LPS-induced macrophage metabolic functions, and altered sensitivity to bacterial and viral infections in vivo when compared to wild-type controls. Our data provide new insights into cellular and molecular mechanisms by which these SNPs decrease the TLR4 signaling efficiency and offer an experimental approach to confirm or refute human data possibly confounded by variables unrelated to the direct effects of the SNPs on TLR4 functionality. ▲

Richard, K., K. H. Piepenbrink, K. A. Shirey et al. (2021) A mouse model of human TLR4 D299G/T399I SNPs reveals mechanisms of altered LPS and pathogen responses. *J Exp Med* **218(2)**. https://doi.org/10.1084/jem.20200675

In-House COVID-19 Serology Quantitative ELISA Test

Susu Zughaier

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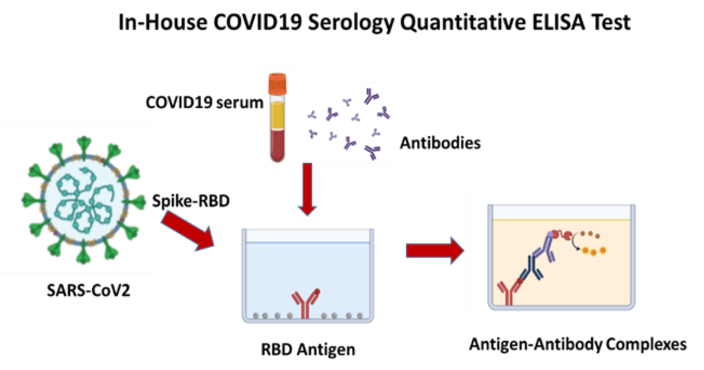
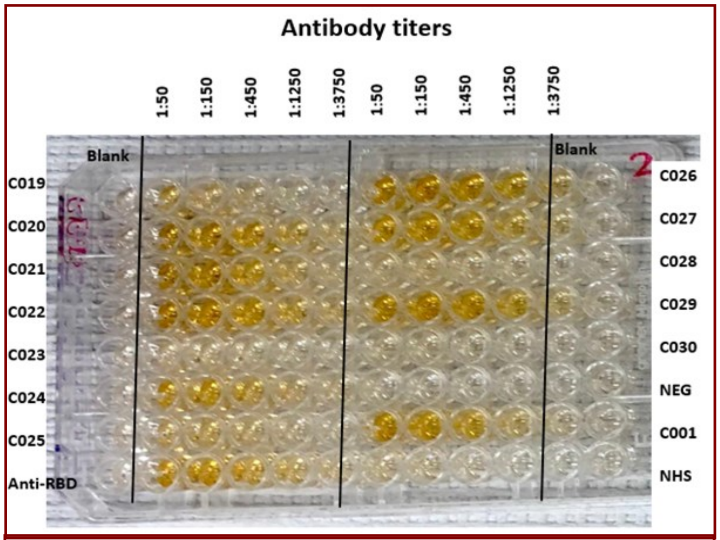
The COVID-19 pandemic created unprecedented demand on supply chain for reagents and diagnostics required to confirm COVID-19 cases. Therefore, development of robust in-house diagnostic tests is of high importance. Upon exposure to infection, host defense mechanisms are activated to curb the infection and initiate protective immune responses. Within few days of exposure, the human body produces specific antibodies (immunoglobulins) that recognize the surface proteins of the invading SARS-CoV2 virus. These antibodies such as IgM followed by IgG immunoglobulins are produced to attack the virus and prevent it from adhering or infecting other host cells. Therefore, virus-specific immunoglobulins are neutralizing antibodies and their appearance in blood is a good sign of seroconversion or immunity. Higher amounts or titers of antibodies are more protective and correlate with removal or reduced viral load. Diagnostics to detect and quantify antibody titers are referred to as serology tests.

At Qatar University, we developed an in-house COVID-19 serology test where we can measure elicited immunity as antibody levels in infected patients. The in-house serology ELISA is sensitive, specific, and reproducible similar to commercially available kits but at much lower cost. The principle of this serology test is based on classical and most common approach to detect antigen-antibody response called the ELISA method.

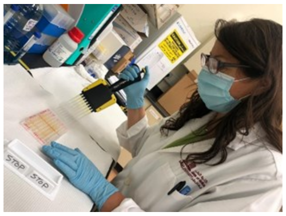


Spike protein is the main surface protein antigen highly expressed in SARS-CoV2. The recombinant spike receptor binding domain (RBD) protein that binds to specific antibodies circulating in COVID-19 patients' blood is used as the antigen in this ELISA test. The serology test is performed in a 96-microtiter well plate coated with RBD protein, therefore, when adding patients' serum to the plate, specific antibodies bind to RBD and form antigen-antibody complexes in the plate. The formed complexes are detected using a secondary antibody labeled with an enzyme tag that gives color proportional to the amount of measured antibody responses. Accordingly, at least 80 serum samples can be screened using one ELISA plate. To measure the antibody titers, 14 serum samples that are serially diluted, can be tested in one ELISA plate in addition to positive and negative controls. This in-house quantitative serology can further measure the type of antibodies (immunoglobins) such as IgM, IgG and IgA, which will give insight on the seroconversion status of COVID-19 patients.



Graphical Abstract: Schematic presentation of an in-house serology quantitative ELISA test

The in-house quantitative serology test is validated using serum samples from severe COVID-19 patients admitted to intensive care at Hamad General Hospital and serum samples from non-COVID-19-infected patients as well. This in-house serology test can help identify convalescent plasma donors i.e. the recovered patients with high titer of antibody response that can be used to treat other patients. This in-house quantitative serology test is much cheaper than commercially available diagnostics and can address the rising issue of COVID-19 supply chain globally in addition to fostering capacity-building efforts at Qatar University. ▲



Acknowledgement: This project is funded by an internal grant from Qatar University (QUERG-CMED-2020-2). This work is in collaboration with Dr. Ali Hussain, intensivist at HMC, Dr. Michail Nomikos at College of Medicine and Biomedical Research Center (BRC) team at Qatar University. Special thanks to Shazeda Chowdhury, Sara Taleb and Mahmoud Elgamal for technical assistance.

IN THE NEWS

Derivatizing LPS for Lipid A Mapping within Tissue


Alison Scott

by Alison Scott, Research Assistant Professor
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We recently published an effective method that allows for the mapping of lipid A directly from LPS within tissue. Using thin tissue sections, we developed an on-tissue chemical derivatization that hydrolyzes the glycosidic linkage between core and lipid A to detect and map lipid A using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). MALDI-MSI is used widely for the generation of molecular maps created from tissue sections, including biomolecules and pharmaceuticals. This technique extends our previous work (Scott et al. 2017 PNAS PMID: 29109289) that demonstrated that lipid A from a bacterial strain bearing predominantly free lipid A (*Francisella novicida*) could be mapped in infected tissues, unlabeled and without chemical modification. While this was an exciting advance, we sought to modify the method to map lipid A from LPS, rather than free lipid A. The challenge for the present work where we aimed to map lipid A from smooth LPS centered on liberating the extensive LPS carbohydrate content from lipid A to allow detection according to our previously established method – a method we named LPS-to-Lipid A-MSI, or LLA-MSI.

In this foundational study, we modified the Fast Lipid Analysis Technique (FLAT) for ultra-rapid lipid A structural screening from Sorensen et al. (2020 Sci Rep, currently under revision) for two-dimensional application to thin tissue sections on glass. In short, a citric acid buffer is applied to the sample, incubated in wet heat, coated with matrix, and analyzed by MALDI. *E. coli* and *P. aeruginosa* spotted cultures were used in dilution series to validate the dose responsiveness of LLA-MSI and the same dilutions evaluated on tissue backgrounds. The key experiment came from mouse lung tissues that were pre-loaded with *P. aeruginosa* culture, treated by LLA-MSI, and analyzed using accurate mass MALDI-MSI. *P. aeruginosa* lipid A was detected in the positive control lung tissues treated with the FLAT chemistry, mapped throughout the tissue area, and was fully resolved from a mouse cardiolipin ion of similar mass.

Studies are underway to map the diverse *P. aeruginosa* lipid A modifications associated with lung infection in mouse models that mimic the airway environment in individuals with cystic fibrosis (CF). We expect that LLA-MSI will yield the first direct, unlabeled description of lipid A structural modification within infected lung tissue. LLA-MSI is the culmination of numerous attempts to use surface chemistries to detect lipid A from smooth LPS on-tissue and wouldn't have been possible without the talents and surface-functionalization background of Hyojik Yang, PhD, the study's lead author. ▲

See publication: **On-Tissue Derivatization of Lipopolysaccharide for Detection of Lipid A Using MALDI-MSI**

Hyojik Yang, Courtney E. Chandler, Shelley N. Jackson, Amina S. Woods, David R. Goodlett, Robert K. Ernst, and Alison J. Scott. *Analytical Chemistry* 2020 **92 (20)**, 13667-13671. DOI: https://10.1021/acs.analchem.0c02566 PMID: 32902263

IEIIS NOW ON SOCIAL MEDIA

Dear Members,

In an effort to expand the outreach and enhance the awareness of our society to all related professionals, we have created several social media accounts as listed below. We are currently in the process to rejuvenise and update the contents, so any inputs and ideas to boost social exposure using these media are highly welcomed. Contributing high quality images from past meetings will be appreciated.

Please contact Hongpeng Jia at: hjia4@jhmi.edu if you can help with the ongoing effort

https://www.facebook.com/pg/International-Endotoxin-and-Innate-Immunity-Society-408088806381204/posts/?ref=page_internal

https://twitter.com/ieiisorg

https://www.linkedin.com/company/international-endotoxin-and-innate-immunity-society/

Fast Lipid Analysis Technique (FLAT) for Microbial Identification and Colistin Resistance Detection via MALDI-TOF MS


Bob Ernst

Bob Ernst, Professor and Chair
Dr. Paul and Mrs. Jean Corcoran, Endowed Professor
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Rapid administration of appropriate antimicrobial therapy significantly improves patient survival, making rapid microbial diagnostics a critical need. We have demonstrated previously that microbial lipids can be sensitive biomarkers for identifying bacterial and fungal species and specific antimicrobial resistance mechanisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (PMID: 28743946). However, current lipid extraction methods require significant hands-on time and are thus not suited for direct adoption as a clinical assay for microbial identification. Here, we report a recently developed method for lipid extraction directly on the surface of stainless-steel MALDI-TOF plate, termed Fast Lipid Analysis Technique (FLAT), which facilitates the identification of bacterial and fungal backgrounds using a sub-60-minute workflow. As of this writing in November 2020, this new method is in revision at *Scientific Reports*.

In order to detect bacterial and fungal lipids, microbial colony smears or liquid samples are applied to a target location on a stainless steel MALDI plate. The target plate is then incubated in a humidified chamber for 30 minutes at 110ºC. The MALDI plate was briefly washed with deionized water, allowed to air dry, and then subsequently 1 uL of norharmane matrix solution was applied to each target location. Spectra were acquired from target locations in negative ion mode using a microflex LRF MALDI-TOF MS in reflectron mode with a limited mass range of 1,000 – 2,400 *m/z*. Total processing time, from the start of sample handling to the end of mass spectra acquisition, was less than 1 hour in all cases.

We initially applied the FLAT method to ~70 bacterial strains that included unique clinical and laboratory adapted isolates of Gram-negative bacteria (*E. coli, K. pneumoniae, A. baumannii, P. aeruginosa, M. morganii,* and *S. marcescens*) including ESKAPE isolates transformed with the *mcr-1* plasmid19, Gram-positive bacteria (*S. aureus, B. cereus,* and *B. mycoides*), and *Candida auris*. We compared FLAT results to results with lipid microextraction (El Hamidi et al; PMID: 19017615) on 157 replicates of the same and similar strains of the same bacterial and fungal species. We also compared MALDI spectra produced by FLAT extraction to the spectra produced by lipid microextraction to determine if the methods extract similar lipid profiles with identical microbial cultures. The results from the two methods produce highly similar spectra with the same characteristic ions.

Lipid spectra are uniquely suited to identifying colistin (polymyxin E) resistance-associated ions. Therefore, we evaluated FLAT as a rapid antimicrobial resistance assay, using bacterial strains that are either intrinsically colistin-resistant or have colistin resistance conferred through acquisition of a plasmid expressing the *mcr-1* gene. This analysis detected phosphoethanolamine (PEtN) or aminoarabinose (Ara4N)-modified lipid A species in all isolates evaluated while also providing species-level identification information in a single assay. Taken together, FLAT has the ability to identify both bacteria and fungal isolates, while simultaneously detecting antimicrobial resistance signals that are not reported by current clinical MALDI ID systems, thus demonstrating the significant potential of FLAT in a clinical diagnostic laboratory setting. ▲

Rapid microbial identification and colistin resistance detection via MALDI-TOF MS using a novel on-target extraction of membrane lipids. *Sci Rep* **10(1)**, 21536. https://doi.org/10.1038/s41598-020-78401-3

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How Do Cells Die? NINJ1 is a New Player

by Alexandre Dinis[1] and Egil Lien[1,2]

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Egil Lien

Cells live and then die, as a natural part of development, but also after cytotoxic insult. We have learned a lot about new aspects of cell death processes over the last 15 years. In addition to apoptosis, which has been studied for decades, we now know much more about autophagy and inflammatory forms of cell death, such as inflammasome-driven pyroptosis and RIPK/MLKL driven necroptosis. There are also several other specialized types of cell death which are less well described. The understanding of the precise mechanisms that lead to the different forms of cell death are still in development. Apoptosis, for example, can be triggered by two signaling cascades, both leading to activation of caspase-3/7: 1) extrinsic apoptosis with caspase-8 as an initiator caspase, and 2) intrinsic apoptosis, which often involves mitochondrial regulators and caspase-97 (Ref 1). We now understand that pyroptosis is driven by caspase-1/11/8-driven limited proteolysis and membrane oligomerization of members of the gasdermin family of proteins, most prominently Gasdermin D (GSDMD). Oligomerization of the N-terminal GSDMD fragments lead to the formation of a membrane pore, which is proposed to be 12-20Å in diameter by different groups. This allows for the passage of small cytokines (such as IL-1β, IL-18), water, ions and other small molecules. The formation of many such pores will increase cytokine release and the influx of water, potentially leading to cell swelling and eventually larger membrane ruptures and cell disintegration (Ref 1). Likewise, the activation of the pseudo-kinase MLKL by RIPK3, often activated by the related kinase RIPK1, leads to membrane permeabilization (Ref 1). Membrane repair systems, like the ESCRT system, can try to counteract damage stemming from pyroptosis and necroptosis, perhaps by "snipping off" membrane areas that are damaged by a pore pattern and thereby preventing the formation of larger membrane ruptures. However, if the death signals are strong, the equilibrium will likely

(Continued on page 10)

How Do Cells Die? NINJ1 is a New Player, *continued*

(Continued from page 9)

shift in favor of these large membrane breaks, leading to the release of larger molecules, such as inflammatory mediators HMGB1, IL-1α and others, than those that can passage via the smaller pores.

Now another piece of the cell death puzzle has been uncovered. Data from Kayagaki, Dixit and colleagues at Genentech suggest in a pre-print (Ref 2) that a specific molecule called NINJ1 (Ninjurin1) is participating in the formation of those larger membrane ruptures. This indicates that the rupture process can be carefully regulated, rather than just driven mechanically by the swelling of a cell. The authors screened macrophages from mutagenized mice for LPS-induced- caspase-11 inflammasome-driven LDH release in order to identify NINJ1 as a key protein. The cytosolic enzyme LDH, often used in cell death assays as a marker of cell permeability when released into supernatants, is too large to exit the cell via GSDMD pores, similar to the inflammatory proteins HMGB1 and IL-1α. In contrast, both LDH, IL-1α and HMGB1 can be released in a process regulated by NINJ1 (Ref 2) (Figure 1). Smaller cell death assay molecules, such as PI and sytox dyes, can also passage via GSDMD pores. The NINJ1-regulated ruptures appear to develop later than the earlier formed smaller pores. Thus, if you work with cell death, it is important to choose assays and time points that take these findings into account. The deletion of NINJ1 in mice led to increased susceptibility to enteric infection with Citrobacter, indicating a key role in host defenses to infection (Ref 2). The involvement of NINJ1 after treatment with stimulators of some inflammasomes and apoptotic processes seems clear, however, data are more complex for necroptotic death. The exact order of events leading to activation of NINJ1 will also have to be worked out, as well as determining the NINJ1 contribution in other types of cell death. Complex formation of NINJ1 is believed to underlie the rupture effect on cell membranes, but the precise reason for this appears unclear. Kayagaki et al. may provide more information in a final version of the paper. These new data also may be evaluated in the context of earlier publications suggesting adhesion molecule (Ref 3) and chemotactic (Ref 4) properties of NINJ1. Nevertheless, these results on regulated membrane ruptures are exciting and should lead to new studies, and to a deeper understanding of the complex mechanisms involved in the demise of a cell and the associated inflammation. ▲

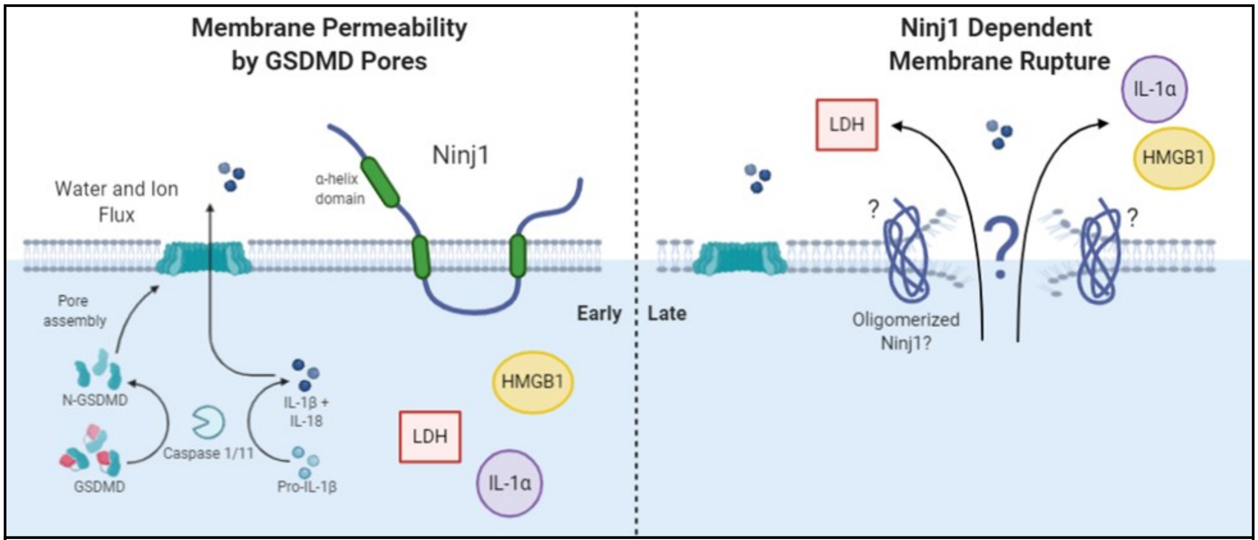

Figure 1: NINJ1 regulates larger plasma membrane ruptures during cell death processes. These ruptures allow for the release of larger molecules such as IL-1α, HMGB1 and LDH. The precise mechanisms are still being worked out. Smaller molecules can flow through more narrow membrane pores, such as those generated by GSDMD.

REFERENCES:

1 Orning, P., Lien, E. (2020) Multiple roles of Caspase-8 in cell death, inflammation, and innate immunity. *J Leuk Biol.* https://jlb.onlinelibrary.wiley.com/doi/abs/10.1002/JLB.3MR0420-305R. DOI: 10.1002/JLB.3MR0420-305R

2 Kayagaki, N., Kornfeld, O.S., Lee, B.L., Stowe, I.B., O'Rourke, K., Li, Q., Sandoval, W., Yan D., Xu, M., Ulas G., Payandeh., Roose-Girma, M., Modrusan, Z., Reja, R., Sagolla, M., Webster, J.D., Cho, V., Andrews, T.D., Morris, L.X., Miosge, L.A., Goodnow, C.C., Bertram, E., Dixit, V.M. (2020) Preprint. Research Square. https://www.researchsquare.com/article/rs-62714/v1. DOI: 10.21203/rs.3.rs-62714/v1

3 Lee, H.J., Ahn, B.J., Shin, M.W., Jeong, J.W., Kim, J.H., Kim, K.W. (2009) Ninjurin1 mediates macrophage-induced programmed cell death during early ocular development. *Cell Death Differ*, **16**:1395

4 Ahn, B.J., Le, H., Shin, M.W., Bae, S.J., Lee, E.J., Wee, H.J., Cha, J.H., Park, J.H., Lee, H.S., Lee, H.J., Jung, H., Parak, Z.Y., Park, S.H., Han, B.W., Seo, J.H., Lo, E.H., Kim K.W. (2012) The N-terminal ectodomain of Ninjurin1 liberated by MMP9 has chemotactic activity. *BBRC* **428**:438

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