ELSEVIER

Institut Pasteur

Microbes and Infection xx (2012) 1–5

www.elsevier.com/locate/micinf

Meeting report

Meeting report: a symposium on the evolution of common molecular pathways underlying innate immunity

Murray B. Gardner [a,*], Nicole Baumgarth [a], Andy Fell [b], Stephen J. McSorley [a], Jay V. Solnick [a,c,d], Charles L. Bevins [d]

[a] *Center for Comparative Medicine, School of Medicine, University of California, Davis, CA 95616, USA*
[b] *Office of University Communications, School of Medicine, University of California, Davis, CA 95616, USA*
[c] *Department of Medicine, School of Medicine, University of California, Davis, CA 95616, USA*
[d] *Department of Microbiology and Immunology, School of Medicine, University of California, Davis, CA 95616, USA*

Received 29 March 2012; accepted 3 April 2012

Abstract

The University of California, Davis hosted a symposium on innate immunity in January 2012. Professors Bruce Beutler, Jules Hoffmann, Luke O'Neill and Pamela Ronald discussed their research on mechanisms that multicellular organisms use to recognize microbes.

Keywords: Innate immunity; Pathogen associated molecular patterns; Pattern recognition receptor; Toll-like receptor; Plant resistance; Drosophila

1. Introduction

One medicine. This theme weaves through many schools and interdisciplinary programs at the University of California Davis. An appreciation that health and disease of both humans and many other mammals are rooted in common biology is perhaps best illustrated in the decade old Center for Comparative Medicine (CCM), a joint venture between the UC Davis Schools of Medicine and Veterinary Medicine. The research mission of the CCM is to investigate host–agent interactions and to develop intervention strategies for persistent infectious diseases common to humans and animals. The CCM provides a strong scientific link between human and veterinary medicine and biomedical research on the UC Davis campus, and extends comparative medicine well beyond the traditional boundaries.

Each year the CCM sponsors a scientific symposium (http://ccm.ucdavis.edu/immunity.html) generously supported by Dr. Murray Gardner, a founding member of the CCM, and Professor Emeritus and former Chair of the Department of Pathology at UC Davis School of Medicine. This year's symposium, held on January 25, 2012, gathered four leading scientists in the field of innate immunity, whose work on rice, flies, mice and humans has established common principles that multicellular organisms use to recognize microbes (Figs. 1 and 2).

2. Inception of the symposium – coincidence rules

The inspiration for this symposium came to Dr. Gardner about a year ago as he was reading a review article entitled "Plant and Animal Sensors of Conserved Microbial Signatures" by Professors Pamela Ronald and Bruce Beutler [1]. The paper struck home because Prof. Ronald had done her PhD research in the Plant Pathology Departments at UC Berkeley and UC Davis. From 1930–1959 Prof. Gardner's father, Dr. Max Gardner was the Chair of this Department at Berkeley, and in 1930 he, together with Dr. James Kendrick, founded the department at Davis., the department Prof. Gardner's father, Dr. Max Gardner had co-founded along with Dr. James Kendrick in 1930.

"While I was growing, up my dad had often told me, although it mostly went in one ear and out the other, that plants

* Corresponding author.
E-mail address: mbgardner@ucdavis.edu (M.B. Gardner).

1286-4579/$ - see front matter © 2012 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2012.04.001

Fig. 1. Participants in the "A Symposium on the Evolution of Common Molecular Pathways Underlying Innate Immunity." A. Participants included from left to right Profs. Bruce Beutler, Jules Hoffmann, Luke O'Neill, and Pamela Ronald, with Dr. Murray Gardner. After introductory comments by Prof. Gardner (B), each speaker gave a 45 min lecture: Prof. Ronald (C, "The Rice XA21 Receptor Recognizes a Conserved Bacterial Signaling Molecule"), Dr. Hoffmann (D, "The Drosophila Host Defense: A Model for the Study of Innate Immunity"), Dr. Beutler (E, "Creating Immune Deficiencies by Random Mutagenesis in Mammals"),and Dr. O'Neil (F, "Toll-like Receptors and Inflammasomes: Key Drivers of Inflammatory Diseases"). Recorded records of each talk can be retrieved at http://ccm.ucdavis.edu/immunity.html.

could teach us something about animal biology and this article proved he was right," Gardner said in his opening remarks at the symposium.

Gardner therefore asked Dr. Ronald to co-organize a symposium on this subject. She suggested including Drs. Bruce Beutler and Jules Hoffmann, who were working on similar receptors in mice and flies, respectively, and they immediately and enthusiastically agreed to take part. Little did the organizers suspect that Beutler and Hoffmann would "detour via Stockholm" on their way to UC Davis [2]. Needing a speaker to cover these receptors in relation to human disease, Dr. Gardner tracked down Dr. Luke O'Neill on the Internet, watched his YouTube video [3] and invited him – not knowing he was simultaneously being invited to UC Davis to give the esteemed Storer Lecture at the same time.

By yet another remarkable coincidence, Pam Ronald and Bruce Beutler are related. Their fathers (Robert Rosenthal and Fred Beutler) were young cousins in Berlin in the 1920s and fled the Nazis in the mid-1930s. Although Ronald knew Bruce's grandmother Kathe well (and her son used the same German crib as three generations of Beutlers and Ronalds), as strange as it may seem, Ronald and Beutler, both living in California, did not know each other and were unaware that they were working for the past 15 years on the same kind of receptor in rice plants and lab mice, respectively. When Ronald realized this coincidence just several years ago, she collaborated with Beutler on the review paper that attracted Gardner's attention and led to the symposium.

3. Plant recognition of pathogens – listening to the conversations of enemies

Much of Ronald's research over the last two decades has helped define how plants detect pathogens [4], and the insights

Please cite this article in press as: M.B. Gardner, et al., Meeting report: a symposium on the evolution of common molecular pathways underlying innate immunity, Microbes and Infection (2012), doi:10.1016/j.micinf.2012.04.001

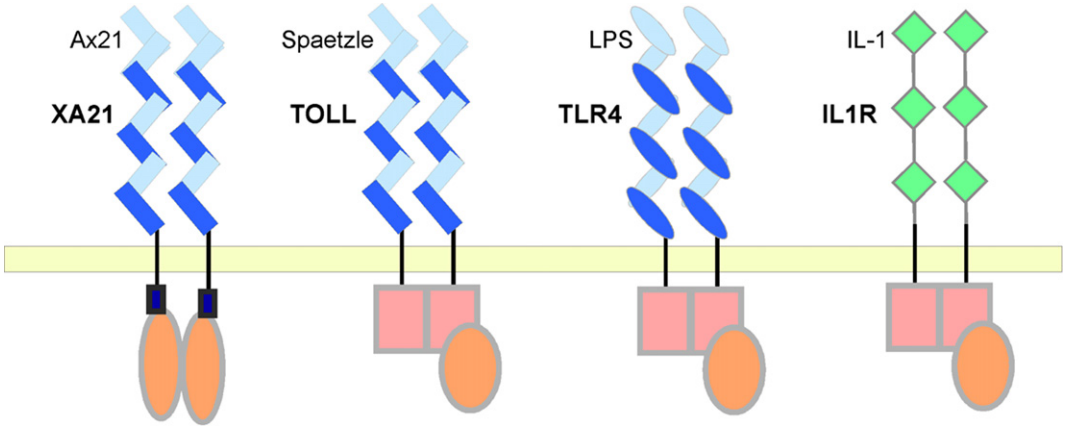

Fig. 2. Domain structure of innate immune receptors discussed at the "A Symposium on the Evolution of Common Molecular Pathways Underlying Innate Immunity." From left to right: Xa21 from rice, Toll from Drosophila, TLR4 from mice and IL-1R from humans. The figure was modeled after an illustration posted by Dr. Ronald at http://phylogenomics.blogspot.com/2012/01/cracking-microbial-code-pam-ronald.html.

from her work now clearly have implications across biology and into the crosshairs of the one medicine concept. Her focus, going back to her postdoctoral fellowship in Dr. Steven Tanksley's laboratory at Cornell University, has been on the genetics and biology of Xa21, a genetic locus that confers resistance for rice plants to the pathogen *Xanthomonas oryzae* pv. *oryzae*. Not only does rice have importance as a staple food, but also when Ronald began her line of investigation it was emerging as a valuable model plant system. Curiously, *Xa21* conferred broad-spectrum resistance against many races of *Xanthomonas*, an unusual property for resistance genes in plants. In 1992, she hypothesized that Xa21 recognized a conserved microbial determinant [5]. Prof. Ronald was unaware that animal biologists were seeking similar receptors of conserved microbial signatures, and commented, "many plant biologists were clueless about the innate immune response of animals. We thought that animals used antibodies and that was it. So we did not read the animal literature as much as we should have (and visa versa…)."

After genetic mapping of this important genetic locus, Ronald came to UC Davis to begin her faculty career. Using a positional cloning approach, in 1995, she and her post-doctoral fellows at UC Davis successfully isolated *XA21*, a single gene encoding the first receptor kinase to be cloned from plants and the first receptor of conserved microbial signatures isolated from plants or animals [6]. In 1996, by the time TOLL was shown to confer an immune response, several other plant immune receptors had been cloned in addition to XA21. Thus, the cloning of *TOLL* was an exciting time for plant biologists, because it showed for the first time that animals responded to infection using receptors similar to those that had recently been isolated from plants [4].

The next challenge for the Ronald lab was to isolate the bacterial ligand recognized by XA21. Using a painstaking proteomics approach, Ronald identified Ax21 as the cognate ligand for Xa21 [7]. Through several years of genetic and biochemical investigation, the Ronald lab had hypothesized that Ax21 was a small, sulfated protein secreted outside the bacterial cell via a type 1 secretion system. The research paid off, as the identification and subsequent characterization of Ax21 yielded the first isolation of a sulfated protein from bacteria and the first description of a post-translational modification (tyrosine sulfation) controlling specificity of the host immune response. Ax21 is highly conserved in all *Xanthomonas* species as well as in *Xylella* and the human pathogen *Stenotrophomonas maltophilia* [7]. Perhaps even more remarkably, Ronald and her colleagues have discovered that Ax21 has quorum sensing activity, a process of information sharing between bacteria via secretion of molecules that coordinates the biological responses of the population and informs bacterial density [8]. Peptide-mediated quorum sensing was previously thought to be restricted to Gram-positive bacteria, so this discovery in *Xanthomonas* represents a remarkable finding [9]. The quorum sensing activity of Ax21, mediated through a newly identified bacterial histidine kinase, is required for biofilm formation and other essential biological activities.

Together these findings on Ax21 suggest for the first time that a bacterial protein serves as a ligand for both plant and bacterial receptors. Furthermore, it appears that rice has evolved to detect a conserved bacterial signaling molecule that is vital to cell–cell communication and virulence of its most important pathogens. This explains the broad-spectrum immune response conferred by *XA21* that originally led Ronald to focus on this genetic locus.

As outlined by the other symposium speakers, subsequent discoveries in flies, mice and humans have revealed that membrane-anchored receptors structurally similar to *XA21* play key roles in recognition of microbial signatures and host defense. These receptors are now commonly referred to as pattern recognition receptors. The conserved signaling molecules (eg. LPS and Ax21), are often called pattern associated molecular patterns (PAMPs).

4. Drosophila – a valuable animal model for study of innate immunity

Hoffmann opened his talk by highlighting the critical importance of understanding anti-microbial defenses in insects,

Please cite this article in press as: M.B. Gardner, et al., Meeting report: a symposium on the evolution of common molecular pathways underlying innate immunity, Microbes and Infection (2012), doi:10.1016/j.micinf.2012.04.001

since they represent the largest number of species on earth and play important roles in plant pollination and infectious disease transmission.

Hoffmann's experimental work that ultimately led to the identification of the Toll receptor in Drosophila immunity was a natural continuation of the seminal work by Drs. Pierre Joly (1915–1997) and Hans Boman (1924–2008) that identified anti-microbial peptides in grasshoppers and butterflies [10]. Dr. Hoffmann's group continued this theme by focusing on how the expression of this early immune defense is regulated in Drosophila. The use of the Drosophila model system allowed the application of powerful genetic tools to innate immunity and ultimately uncovered the molecular basis of early host recognition of microbial pathogens [11]. These investigations led to an explosion of research in innate immunity and led to Hoffmann sharing the Nobel Prize in Physiology and Medicine in 2011 [2].

After initially cloning the gene encoding the Drosophila anti-microbial peptide diptericin, Hoffmann noted that it appeared to contain NF-κB response elements and that these sequences were required for diptericin production in response to infection. An NF-κB family member, *dorsal*, was known to be important in Drosophila embryonic development and initiated a signaling cascade that ultimately led to activation of the transmembrane receptor Toll by the cleaved growth factor *spaetzle*. This naturally led Hoffmann to examine whether dorsal was required for diptericin expression in response to immune activation, but no role for dorsal in diptericin induction could be found. Subsequent proteomic analysis of infected flies then led to the identification of drosomycin, an anti-microbial peptide that showed little activity against bacteria but was active against fungi. This breakthrough was important since Hoffmann's group found that drosomycin expression required Toll pathway signaling while a second signaling pathway requiring the "immune deficiency" imd gene regulates diptericin expression in response to infection.

Importantly, Drosophila harboring mutations in the Toll pathway were highly susceptibility to *Aspergillus* infection, while the imd pathway was shown to be required for resistance to *E. coli* infection [12]. The seminal observations of this landmark paper, published in 1996 therefore uncovered innate signaling pathways that are required for early anti-microbial defense in insects and complementary studies demonstrated the importance of similar pathways in plant and mammalian innate immune defense.

Hoffmann highlighted the similarity between Drosophila and mammalian, Toll and Toll-like receptor (TLR), IMD and TNF-α receptor signaling pathways, all converging on the host NF-κB pathway. The evolutionary conservation of these modules of innate immune activation through vertebrate and mammalian evolution emphasizes the importance of these pathways in providing early immune defense even in organisms with more complex adaptive immune capabilities.

5. Mice – the power of genetics

Beutler began his presentation by outlining his long-standing interest in the effects of lipopolysaccharide (LPS) on mammals, a structural bacterial component of gram-negative bacteria first identified as "endotoxin" by R. Pfeiffer in the late 1880's. Identification of its receptor over 110 years later, of course, won Beutler a share of the Nobel Prize in Physiology and Medicine in 2011 [2].

During his early studies with Dr. Anthony Cerami at the Rockefeller University, Beutler isolated "cachectin", now know as tumor-necrosis factor (TNF-α), as an important factor secreted by LPS-stimulated macrophages from C3H/HeN mice, but not from the related C3H/HeJ strain, which they reported in 1986. C3H/HeJ mice were known to be relatively endotoxin-resistant, due to a likely single-gene mutation that in the late 1970's had been linked to mouse chromosome 4. These findings laid the groundwork for identifying TLR4 as the mouse LPS receptor.

Using the mouse genetic tools available at that time, Beutler embarked on isolating the *lps*^{d} gene in C3H/HeJ mice. Cross-over mutant mice located the gene within a 2.6 Mb region on chromosome 4, which his lab began to sequence around 1993. By 1997 the group had ruled out over 90 percent of that region, finding mainly pseudogenes, when they were struck by the presence of the TLR4 gene in the remaining region on chromosome 4. TLR4 was an appealing candidate, since Drosophila Toll mutations were just shown by Hoffmann and colleagues to increase susceptibility to infections in flies. Moreover, the ectopic domains of TLR4 were remarkably similar to CD14, a known LPS-binding factor that lacked a signaling chain, while its intracellular structure was similar to that of the IL-1R and Drosophila Toll. The landmark paper of 1998 [13], and the key follow-up study subsequently published in 2000 [14] provided the irrefutable genetic evidence that TLR4 was clearly a receptor for LPS of animal innate immunity, and not a receptor functioning in developmental pathways, which at the time was an equally plausible notion based on early studies in flies. Further studies identified the TLR4/MD2 complex as the LPS-receptor capable of activating NF-κB and causing the release of TNF-α from macrophages [15]. Subsequent work, particularly by Shizuo Akira led to the rapid identification of ligands for the other TLR and the concept of "pattern recognition receptors".

In the second part of his talk, Beutler outlined his current "forward genetics" screening approach via chemically (ENU) induced-mutations of mice [16]. His focus is on identifying genes affecting humoral immunity, pattern-recognition receptor signaling, resistance to MCVM infection and intestinal homeostasis, where the group is interested in determining the genetic etiology underlying inflammatory bowl disease (IBD).

IBD is thought of as a "complex" disease in humans with a clear genetic basis, as established in human twin studies. Beutler addressed the question of whether IBD was truly a "complex" genetic disease, involving multiple affected genes in humans, or whether it is a disease in which mutations in a single gene could give the phenotypic picture of IBD, but in which different genes might be affected in different patients.

Using his current library of over 5800 ENU-mutated mouse lines, Beutler finds much support for the "one mutation away" hypothesis. To date, his group has identified mutations in 22 genes, each of which generates an IBD phenotype. Among the

Please cite this article in press as: M.B. Gardner, et al., Meeting report: a symposium on the evolution of common molecular pathways underlying innate immunity, Microbes and Infection (2012), doi:10.1016/j.micinf.2012.04.001

affected genes they find genes affecting epithelial cell homeostasis and repair in the gut, such as defects in the endoplasmic reticulum, stress responses or proliferation. Such mutations would be expected to ultimately break the epithelial barrier and enable gut microbes to invade the mammalian host. Other mutations that cause a similar phenotype seem to be affecting primarily the hematopoietic cell system, in particular the regulation of the innate immune responses that usually control microbial infections. Based on current data, Beutler concluded that there might be roughly 130 genes in the human genome that when mutated could cause Crohn's disease, explaining its relatively high prevalence.

6. Humans – last, but not least

O'Neill began his scientific career in the 1980s working on prostaglandins and IL-1 signaling. He was in the right place at the right time: the discovery that the IL-1 receptor was related to the insect Toll, and to the product of the tobacco mosaic virus resistance gene N, led to the realization that there were highly conserved sensor molecules across all kingdoms of life—what we now know as the Toll-like receptors, which serve to sense PAMPs and danger signals from host tissue damage [17]. Much of his career has been devoted to understanding TLR mediated signaling pathways, but more recently he has also begun to ask whether it can be put to clinical use. Inhibitors of downstream cytokines such as TNF-α and IL-1 are already in clinical practice for inflammatory diseases [18].

Perhaps, O'Neill reasoned, since the role of TLRs is broadly to detect danger signals, blockade at the level of the TLR may be more effective. Studies published last year showed that *ex vivo* antibody blockade of TLR2 could markedly reduce IL-6 and TNF-α in tissue from patients with rheumatoid arthritis. Many other inflammatory diseases are also mediated by TLR2, including ischemia reperfusion injury that complicates cardiac surgery and kidney transplantation. Preliminary experiments in mice and in pigs demonstrated that antibody to TLR2 could in fact prevent the acute and chronic effects of reperfusion injury. These and others findings have led to the rapid translation of the groundbreaking earlier work in rice plants, Drosophila and mice into new therapeutic applications. A Phase 1 clinical trial in partnership with Opsona Therapeutics has now demonstrated the safety of humanized anti-TLR2 in humans; a Phase 2 clinical trial will begin in kidney transplant patients in June. Finally, O'Neill argued that not only infection and tissue damage generate inflammation, but also metabolic disturbances that engage the inflammasome, leading to production of IL-1. In the end, inflammatory diseases likely result from one or more genetic susceptibilities, which provide a tipping point for a maladaptive response to infection or other environmental insult.

7. Concluding comments

A remarkable set of circumstances converged to bring this Symposium to the UC Davis campus. A treat for all who could attend this interdisciplinary symposium, each presentation of the Symposium was thought provoking, inspiring, and entertaining. The research highlighted by the Symposium speakers demonstrated how a remarkable evolutionary conservation of innate immune mechanisms has become apparent between flies, plants, mice, and humans. Each of these species uses similar receptors to detect microbes. All four speakers demonstrated that unifying themes of biology can converge to fuel an appreciation for "one medicine." Therapeutic targeting of toll-like receptors for infectious, inflammatory and neoplastic diseases, and crop engineering of these receptors for resistance to infection in plants are now becoming a reality.

Audio recordings of the Symposium are available at the UC Davis CCM website http://ccm.ucdavis.edu/immunity.html.

References

[1] P.C. Ronald, B. Beutler, Plant and animal sensors of conserved microbial signatures, Science 330 (2010) 1061–1064.
[2] http://www.nobelprize.org/nobel_prizes/medicine/laureates/2011/press.html.
[3] http://www.youtube.com/watch?v=ej3KLitdKtw&noredirect=1.
[4] B. Schwessinger, P.C. Ronald, Plant innate immunity: perception of conserved microbial signatures, Annu. Rev. Plant Biol. 63 (2012) 451–482.
[5] P.C. Ronald, B. Albano, R. Tabien, L. Abenes, K.S. Wu, S. McCouch, S.D. Tanksley, Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*, Mol. Gen. Genet. 236 (1992) 113–120.
[6] W.Y. Song, G.L. Wang, L.L. Chen, H.S. Kim, L.Y. Pi, T. Holsten, J. Gardner, B. Wang, W.X. Zhai, L.H. Zhu, C. Fauquet, P. Ronald, A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*, Science 270 (1995) 1804–1806.
[7] S.W. Lee, S.W. Han, M. Sririyanum, C.J. Park, Y.S. Seo, P.C. Ronald, A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity, Science 326 (2009) 850–853.
[8] S.W. Han, M. Sriariyanun, S.W. Lee, M. Sharma, O. Bahar, Z. Bower, P.C. Ronald, Small protein-mediated quorum sensing in a gram-negative bacterium, PLoS One 6 (2011) e29192.
[9] P.C. Ronald, Small protein-mediated quorum sensing in a gram-negative bacterium: novel targets for control of infectious disease, Discov. Med. 12 (2011) 461–470.
[10] K. Putsep, I. Faye, Hans G Boman (1924–2008): pioneer in peptide-mediated innate immune defence, Scand. J. Immunol. 70 (2009) 317–319.
[11] D. Ferrandon, J.-L. Imler, C. Hetru, J.A. Hoffmann, The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections, Nat. Rev. Immunol. 7 (2007) 862–874.
[12] B. Lemaitre, E. Nicolas, L. Michaut, J.M. Reichhart, J.A. Hoffmann, The dorsoventral regulatory gene cassette spaetzle/Toll/cactus controls the potent antifungal response in Drosophila adults, Cell 86 (1996) 973–983.
[13] A. Poltorak, X. He, I. Smirnova, M.Y. Liu, C.V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, B. Beutler, Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene, Science 28 2 (1998) 2085–2088.
[14] A. Poltorak, P. Ricciardi-Castagnoli, S. Citterio, B. Beutler, Physical contact between lipopolysaccharide and Toll-like receptor 4 revealed by genetic complementation, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 2163–2167.
[15] B. Beutler, E.T. Rietschel, Innate immune sensing and its roots: the story of endotoxin, Nat. Rev. Immunol. 3 (2003) 169–176.
[16] B. Beutler, The Toll-like receptors: analysis by forward genetic methods, Immunogenetics 57 (2005) 385–392.
[17] L.A. O'Neill, The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress, Immunol. Rev. 226 (2008) 10–18.
[18] L.A. O'Neill, C.E. Bryant, S.L. Doyle, Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer, Pharmacol. Rev. 61 (2009) 177–197.

Please cite this article in press as: M.B. Gardner, et al., Meeting report: a symposium on the evolution of common molecular pathways underlying innate immunity, Microbes and Infection (2012), doi:10.1016/j.micinf.2012.04.001