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Editors

The Role of Toll-Like Receptor 4 in Infectious and Non Infectious Inflammation

Springer

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Preface

Toll-like receptors (TLRs) are essential regulators of innate and adaptive immune responses and their complexity continues to intrigue researchers, including the authors of this book. Because of their importance, when they are mutated and not functioning as they should, autoimmune, inflammatory, and infectious diseases can develop.

The aim of this book is to present an update on the role of TLR4, the most studied TLR, in inflammatory and infectious diseases with special focus on central nervous system (CNS) pathologies.

We also give an outlook on what emerged in the past years on the molecular aspects of extracellular TLR4 activation and intracellular signaling, its regulation by miRNA, and crosstalk with other metabolic pathways.

To this end, a group of internationally recognized experts has kindly accepted to present recent results on TLR4 function and role in health and disease.

TLR4 was the first TLR identified by Medzhitov and coworkers in 1997 and was then characterized by Beutler and coworkers as a pattern recognition receptor. TLR4 has an exquisite ligand selectivity that has remained largely unchanged through the course of evolution allowing for an immediate and sensitive response to gram-negative bacterial lipopolysaccharide (LPS).

The understanding of molecular features of extracellular TLR4 activation has contributed to unravel the physiological and pathological role of TLR4. We have now information on the structural biology of the molecular actors of LPS transfer: LBP and CD14 proteins, the M-shaped dimeric $(TLR4\text{-}MD2\text{-}LPS)_2$ complex, and of the intracellular signaling proteins, belonging to the so-called MyD88-dependent and MyD88-independent pathways. The LPS-binding protein CD14 not only takes part in LPS extraction from aggregates in solution and shuttling to TLR4/MD-2/LPS dimer, but it also exerts autonomous functions by regulating endocytic processes or by activating dedicated signaling pathways, as critically reviewed by M. Di Gioia and I. Zanoni.

Starting from the knowledge of the supramolecular interactions among LPS and LBP, CD14 and MD-2, the structure–activity relationship of TLR4 ligands, especially of lipid A variants, has been extensively studied. These studies allow the structure-based rational design of synthetic or semisynthetic lipid A variants as vaccine adjuvants as reviewed by A. Shimoyama and K. Fukase.

Natural TLR4 ligands, LPS and LOS, and their synthetic variants are amphiphilic molecules that aggregate in solution. A. B. Schromm and K. Brandenburg discuss from a biophysical perspective the most recent achievements in the study of the role of aggregates in the biological activity of TLR4 ligands, with special focus on the very recent findings on LPS interaction with intracellular caspases and subsequent induction of the non-canonical inflammasome.

To complete the complex and fascinating view of TLR4 signaling at a molecular level, N. Kuzmich dissected from the structural biology point of view the two distinct intracellular pathways activated upon TLR4 dimerization: the MyD88-dependent pathway and the TRIF/IRF3 pathway leading to interferon production. He discussed the molecular events including phosphorylation and ubiquitination that allow the regulation of the pathways.

Septic shock or excessive inflammation are possibly the most severe outcomes due to inadequate negative regulation of TLR4 signaling leading to excessive pro-inflammatory cytokine production. Similarly, TLR4 excessive stimulation by endogenous molecules derived from necrotic or damaged tissues (danger-associated molecular patterns, DAMPs) has been associated to a wide array of inflammatory and autoimmune diseases, including neuroinflammations and vascular inflammations.

In this perspective, M. Christodoulides reviewed the molecular mechanism of *Neisseria* lipooligosaccharide (LOS) interaction with TLR4 and discussed the role of TLR4 in meningococcal and gonococcal infections in the therapeutic perspective to target *Neisseria* infections with TLR4 antagonists or investigating the role of TLR4 in *Neisseria*-vaccine-induced immune responses.

On the other hand, M. Molteni and C. Rossetti analyzed the main families of endogenous TLR4 stimulators (DAMPs) and discussed DAMP/TLR4 activation mechanisms that very often differ from the well-known direct TLR4/MD-2 binding of bacterial endotoxins.

Another aspect still underestimated relative to TLR4 biology is the crosstalk between TLR4 signaling and metabolic regulations occurring in macrophages and dendritic cells (DCs). TLR4 stimulation of DCs or macrophages results in increased glycolytic activity, an essential process to support their pro-inflammatory functions. L. Perrin-Cocon, A. Aublin-Gex, and V. Lotteau reviewed the molecular mechanisms involved in the modulation of central carbon metabolism, from glycolysis, tricarboxylic acid (TCA) cycle, and oxidative phosphorylation to lipid metabolism, upon TLR4 signaling in macrophages and DCs.

Molecules called microRNAs (miRNA) have been recently described as negative regulators of TLR signaling acting as a break on the pathway while others act as

positive regulators and act as an accelerator. There is increasing evidence that TLR4 and miRNA crosstalk ensures the fine-tuning of inflammatory response and subsequent healing occurring in tissues after infection or injury. The expression of miRNAs inside the cell, after TLR4 triggering, critically contributes both to the activation and to the shutdown of immune cell response needed for the termination of the inflammatory process. M. Molteni and C. Rossetti gave insight into the intracellular role of TLR4-miRNA axis in the regulation of inflammatory and anti-inflammatory processes.

Interestingly, TLR4 does not only play a role in inducing inflammation but also in inducing tissue regeneration and healing after inflammatory insult, and it maintains homeostasis in the gut and a state of constant "controlled inflammation" due to the stimulation by commensal bacteria. When there is an imbalance of gut microflora, inflammatory bowel diseases can develop.

This dual role of TLR4 signaling is particularly critical in CNS, as discussed by L. De Filippis and F. Peri. TLR4 is expressed in microglia, which is a master player in neuroinflammatory processes, as well as in neural cells: astrocytes, neurons, oligodendrocytes, neural progenitors (NPC), and neural stem cells (NSC). We have observed that TLR4 stimulation by LPS during differentiation enhances neuronogenic potential of human NSC and favors both neuronal and oligodendroglial survival. Consistent with our data, it has been recently reported that endogenous NSC are actively stimulated to proliferate by TLR4 activation after stroke, thus confirming the relevant role of TLR4 in promoting neurogenesis under non-physiological conditions. Altogether, these results indicate that in a therapeutic perspective, TLR4 activity should not to be turned on or off, but should be finely tuned in order to promote neuroregeneration rather than neurodegeneration and to mediate the development of healing immunomodulation rather than of detrimental neuroinflammation.

Recent insight on the role of TLR4 as mediator of inflammatory response in Alzheimer's (AD) and Parkinson's disease (PD) has been reviewed by C. Balducci and G. Forloni. They reported literature data relating activation of TLR4 and the presence of β amyloid and their own data showing that the memory damage and inflammatory effects obtained by intraventricular application of β amyloid oligomers was antagonized by TLR4 inhibitor and completely abolished in TLR4-knockout mice. They also discuss the recently discovered role of TLR4 in the relationship between gut microbiota dysbiosis and increased risk of developing PD.

M. De Paola presented new data on the potential of Human Induced Pluripotent Stem Cells (IPSC) and Cerebral Organoids as models to study how TLR4 regulates immune cell interactions to orchestrate brain development and reaction to injury. He showed how the development of platforms in which microglia, neurons, and macroglia derived from healthy or diseased subjects grow and mature in a single system allows to demonstrate the role of TLR4 in mediating neuroinflammation.

We thank all authors, that are also our good friends, for having accepted the challenge to compose this multidisciplinary mosaic around TLR4 functions. Their different expertise in the fields of structural biology, biophysics, medicinal chemistry,

computational biology, biochemistry, immunology, pharmacology, and medicine made possible the creation of a unique overview on several aspects of physiological and pathological roles of one of the most fascinating and smart molecules to which we have dedicated a large part of our scientific adventure.

Varese, Italy — Carlo Rossetti
Milan, Italy — Francesco Peri

Contents

About the Editors

Carlo Rossetti was born in Brescia in 1958 and graduated in Biological Sciences in 1982. Currently, he is Associate Professor in Applied Biology at the University of Insubria (Varese, Italy). His research focuses on the role of Toll-like receptors in tissue regeneration and activation of the innate immunity. In 2008, he founded a start-up for the development of TLR4-antagonist natural molecules.

Francesco Peri Full Professor (Organic and Medicinal Chemistry) at the Department of Biotechnology and Biosciences of the University of Milano-Bicocca (Milano, Italy). He holds a permanent professorship at the École Normale Supérieure (ENS) of Lyon (France), where he teaches a course of Medicinal Chemistry (Master level). In 2012, he had a permanent professorship in Organic Chemistry at the University Paris 5; and in 2012, he was for 1 year Visiting Professor at the Department of Chemistry, University of California, Davis (USA), giving graduate courses.

His research activity focuses on medicinal chemistry, bioorganic chemistry, and drug development. New TLR4 modulators have been developed by his group, and novel cardiac drug leads modulating the activity of the Serca2a protein.

He has numerous international and national collaborations, holds national and international grants, and is the president of the MicrobiotaMi association, whose mission is to diffuse the knowledge and the culture related to studies on human microbiota. He is committed to technology transfer and is Delegate of the Rector for the University of Innovation Foundation (www.u4i.it), an organization devoted to the valorization of innovation and to decisional support to University start-ups.

He is founder of the academic spin-off CP2 Biotech, whose mission is the valorization of new drug hits and the development of innovative therapeutic approaches to vaccine adjuvants and inflammatory diseases.

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