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Pharmacology and therapeutic potential of pattern recognition receptors

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ABSTRACT

Pharmacologists have used pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) for decades as a stimulus for studying mediators involved in inflammation and for the screening of anti-inflammatory compounds. However, in the view of immunologists, LPS was too non-specific for studying the mechanisms of immune signalling in infection and inflammation, as no receptors had been identified. This changed in the late 1990s with the discovery of the Toll-like receptors. These 'pattern recognition receptors' (PRRs) were able to recognise highly conserved sequences, the so called pathogen associated molecular patterns (PAMPs) present in or on pathogens. This specificity of particular PAMPs and their newly defined receptors provided a common ground between pharmacologists and immunologists for the study of inflammation. PRRs also recognise endogenous agonists, the so called danger-associated molecular patterns (DAMPs), which can result in sterile inflammation. The signalling pathways and ligands of many PRRs have now been characterised and there is no doubt that this rich vein of research will aid the discovery of new therapeutics for infectious conditions and chronic inflammatory disease.

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1. Introduction

Pharmacologists and immunologist have been using pathogen associated molecular patterns (PAMPs) derived from bacteria, viruses and fungi for many years. This is exemplified by the use of lipopolysaccharide (LPS), a component of the Gram negative bacterial cell wall, by pharmacologists as a ubiquitous pro-inflammatory stimulus for investigating immune and vascular responses. LPS challenge in vitro and in vivo has led to the discovery and assessment of the molecular mechanisms of a large number of pro- and anti-inflammatory mediators. Other PAMPs have also been widely used by the pharmaceutical industry for the screening of many anti-inflammatory compounds. As LPS is now known to act on cells through the pattern recognition receptor (PRR), Toll-like receptor (TLR) 4 (Poltorak et al., 1998), we may therefore already attribute the discovery of many novel therapeutic agents in part to the pharmacological manipulation of PRRs.

PRRs are part of the innate immune system and are integral to the switch between innate and adaptive immunity as well as regulating ongoing aspects of the adaptive immune response. This facilitates the final stage, which is killing and removal of the pathogen. It is also worth noting at this point that PRRs not only recognise non-self components, but also recognise endogenous moieties that are not normally exposed. These components become recognisable when the host suffers trauma, which can be caused by infection, chemical

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or physical damage. The innate immune system recognises these components as danger signals, much like the presence of virulent microorganisms. Evidence is emerging that PRRs may function as part of normal homeostatic processes that are activated by physiological levels of, as yet, unknown ligands (Zhang et al., 2006; Harrington et al., 2007). Therefore the immune system has developed not only to recognise and remove invading pathogens but also to identify the tissue damaged caused by these pathogens. Additionally PRRs can be activated inappropriately by innocent host molecules as occurs in sterile inflammation and autoimmunity.

This review will firstly consider how PAMPs have been used in research from a historical perspective prior to the fuller characterisation of PRRs. We will then address how understanding the signalling pathways of PRRs can be used to identify therapeutic targets and biomarkers of a variety of disease states.

2. Historical pharmacological perspective of pathogen-associated molecular patterns

2.1. The effects of lipopolysaccharide on biological systems

LPS is, as the name suggests, a large molecule consisting of a lipid and a polysaccharide bound covalently and is the major constituent of the outer cell membrane of Gram-negative bacteria (Raetz, 1990). Prior to the discovery that LPS binds to TLR4 through a complex series of co-receptor interactions, many used LPS as the archetypal stimulus in inflammatory models. This was done in relative disregard for how LPS induced inflammation. The inflammation LPS provokes, bears many hallmarks of that induced by pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα and produces robust and reproducible responses both in vitro and in vivo. Lipopolysaccharide binding protein (LBP) binds to the highly conserved lipid A portion of LPS (Tobias et al., 1989) and is an acute phase protein produced primarily but not exclusively by the liver (Grube et al., 1994). LBP modulates the innate immune response to Gram negative infection by binding to LPS in the serum and then facilitating binding to the TLR4/MD2 complex which stimulates downstream immunoprotective pathways. LBP knockout mice have been shown to be protected from the effect of experimental LPS administration with regard to cytokine release but interestingly, in infection models, were at increased risk of death (Jack et al., 1997) and so it can be surmised that LBP is required for immunoprotection even if this is not necessarily related to LPS. LBP appears to be capable of binding to molecules other than LPS and has been shown to respond to both Gram negative and positive bacteria (Schroder et al., 2004). It may be that LBP acts as a soluble PRR that recognises and presents bacterial PAMPs to the appropriate TLR (Schumann, 2011).

2.1.1. Different forms of lipopolysaccharide

Although widely recognised as the archetypal TLR4 agonist, it is worth acknowledging that not all forms of LPS are equivalent. For example, LPS from two periodontopathic bacteria, *Porphyromonas gingivalis* and *Capnocytophaga ochracea* are antagonists for human TLR4 (Yoshimura et al., 2002; Darveau et al., 2004) and that LPS from *P. gingivalis* can act as a TLR2 agonist as well as a TLR 4 antagonist (Darveau et al., 2004).

2.2. Manipulation of lipopolysaccharide activated responses for drug discovery and screens

When isolated cell systems are stimulated with LPS in vitro a large gamut of inflammatory and vasoactive genes are induced. The use of LPS has notably led to critical mechanistic advances in the understanding of the role of nitric oxide synthase 2 (Stuehr & Marletta, 1985), cyclooxygenase 2 (Lee et al., 1992), heme oxygenase 1 (Camhi et al., 1995), annexin-1 (Wu et al., 1995), glucocorticoids, CXCL8 (IL-8) (DeForge et al., 1992) and TNF-α (Tracey et al., 1988) in inflammation. Importantly, inflammation induced by LPS in monocytes and macrophages has been exploited by pharmacologists for the development and testing of the potency of two of the most commonly used groups of anti-inflammatory drugs, these being non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (see Fig. 1).

2.3. Lipopolysaccharide activated responses in the vasculature

LPS also targets the vasculature where TLR4 is expressed (Opitz et al., 2009) and mediates vascular dysfunction (Cartwright et al., 2007a) and induction of genes (Jimenez et al., 2005) by Gram negative bacteria. This was highlighted by Lloyd Maclean and Max Weil in 1956, who showed that intravenous injection of LPS in dogs resulted in profound and immediate hypotension, which was in part explained by a severe anaphylactic reaction (Maclean & Weil, 1956). We now know that the hypotension caused by LPS is biphasic, with an initial drop in blood pressure observed in rodents and dogs within minutes of its administration, which seems to be mediated by the acute release of vasoactive and inflammatory mediators. The late phase vascular collapse, which is resistant to vasoconstrictors (Szabo et al., 1993), is associated with nitric oxide synthase II induction within the vascular smooth muscle of blood vessels (Szabo et al., 1993) which can be mimicked by treating vessels in vitro with LPS (Bishop-Bailey et al., 1997). These observations in vivo led to clinical trials for both nitric oxide synthase inhibitors (Petros et al., 1991) and anti-LPS (Ziegler et al., 1991) therapy for sepsis. Interestingly, these were not successful due to a paradoxical increase in cardiovascular events with the nitric oxide synthase inhibitor (546C880) (Lopez et al., 2004) and increased complexity of sepsis due to the presence of polymicrobial infections and bacterial toxins in anti-LPS therapy (HA-1A) (Derkx et al., 1999). Work from our group also highlights the role of other vasoactive mediators in sepsis and suggested that global inhibition of NO is detrimental to the heart (Price et al., 2002, 2003) where induction of endothelin-1 and subsequent activation of constrictor ETA receptors impact on heart cell function (Patel et al., 2007). These trials sound a note of caution for how we should interpret data from both in vitro and in vivo models using purified PAMPs, heat killed bacteria and PRR pharmacology.

2.4. The use of zymosan as a pharmacological tool in acute inflammation

Zymosan is a fungal derived PAMP that has been used for many years by pharmacologists as a tool in the study of inflammatory mediators and in screening for anti-inflammatory compounds. Zymosan is a glucan that is purified from the yeast *Saccharomyces cerevisiae*. When it is injected in vivo, zymosan becomes coated in the serum complement components C3b/C3bi. This activates the complement cascade resulting in the release of the potent leukocyte chemoattractant C5a (Brade & Vogt, 1971; Fearon & Austen, 1977; Stahl et al., 1991). More recently, zymosan has been shown to be an agonist of TLR2 and Dectin-1. Although, Dectin-1 activation is sufficient to induce phagocytosis in macrophages, when these receptors act together they synergise to enhance nuclear factor kappa B activation and to mediate production of cytokines such as interleukin-12 and TNFα (Gantner et al., 2003; Goodridge et al., 2007). However, in some organ systems such as the lung, zymosan has been shown to activate the innate immune system independently of TLRs, complement or Dectin-1 by an as yet undefined mechanism (Kelly et al., 2008). Despite the full mechanism of zymosan-induced inflammation remaining unknown, its pharmacology has been exploited for the study of many novel mediators. Zymosan injected into an air pouch or the peritoneum of mice provides us with a very useful and robust in vivo model for the study of acute resolving inflammation. This acute zymosan model has been used for mechanistic studies to characterise the involvement of annexin-1 (Perretti et al., 1993; Getting