Molecular Immunology 51 (2012) 356–362

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Short communication

"Monovalent" ligands that trigger TLR-4 and TCR are not necessarily truly monovalent

Alexander B. Sigalov*

SignaBlok, Inc., P.O. Box 4064, Shrewsbury, MA 01545, USA

ARTICLE INFO

Article history:
Received 7 March 2012
Accepted 25 March 2012
Available online 20 April 2012

Key words:
Immune signaling
Receptor clustering
Ligand valency
LPSL
Lipid A
Micelles
Toll-like receptor
TLR-4
Surface-bound
MHC
T cell receptor

ABSTRACT

Cell surface receptors mediate many cellular responses in health and disease. Recent progress in our understanding of how ligand binding to the extracellular domains of receptors triggers intracellular signaling has underlined the role of ligand-promoted receptor clustering following by oligomerization of the cytoplasmic signaling domains. The clustering suggests the requirement of ligand multivalency and is especially important for triggering receptors involved in innate and adaptive immune responses. However, although numerous studies have established that multivalent, but not monovalent, ligands induce receptor-mediated signal transduction, considerable uncertainty still remains. Here, I hypothesize that "monovalent" ligands that have been reported to trigger immune receptors *in vitro* are not necessarily truly monovalent. This is illustrated by focusing on studies of signal transduction by toll-like receptor-4 and T cell receptor. By generalizing this concept to a variety of lipid and protein ligands, one would propose an alternative interpretation of apparent ligand monovalency in other receptor activation studies as well.

1. Introduction

Cell activation mediated by membrane receptors that recognize a variety of lipid and protein ligands plays an important role in health and disease. Numerous studies have shown that multivalent, but not monovalent, ligands are required to trigger receptors and initiate signaling cascades (reviewed in detail in Cooper and Qian (2008), Klemm et al. (1998), Sigalov (2004, 2010a), Weiss and Schlessinger (1998)). This raised the question how multivalent ligand binding triggers receptor signaling. A recently suggested general model of immune signaling, the Signaling Chain HOmoOLigomerization (SCHOOL) model (Sigalov, 2010a), provides a possible answer to this question and assumes that receptor clustering induced or tuned upon multivalent ligand binding outside the cell is translated across the membrane into oligomerization and transactivation of cytoplasmic signaling domains. The necessity and sufficiency of formation of competent signaling oligomers to trigger receptor function dictates several important restraints, including sufficient interreceptor proximity and correct (permissive for signaling) relative orientation of the units in receptor dimers/oligomers (Sigalov, 2010a, 2010b). However, despite our improved understanding of transmembrane signal transduction, considerable uncertainty still remains on whether monovalent ligands are able to activate receptors involved in the immune responses.

The requirement of ligand multivalency is apparently questioned in studies of toll-like receptor-4 (TLR-4), the receptor that detects the presence of lipopolysaccharide (LPS) from Gram-negative bacteria and initiates innate immune defensive responses (Akira and Takeda, 2004; Fitzgerald and Golenbock, 2007; Jin and Lee, 2008; Lu et al., 2008; Takeda and Akira, 2005). The complex formed by TLR-4 and the accessory protein MD-2 mediates the recognition of lipid A (Akira and Takeda, 2004; Beutler, 2002), the lipid component of LPS that is associated with toxicity of LPS and has been termed the "endotoxic" principle of endotoxin (Rietschel et al., 1987; Seydel et al., 2003). Ligand-induced and cytoplasmic domain-mediated TLR-4 oligomerization is known to trigger TLR-4 and initiate intracellular signaling cascade (Brodsky and Medzhitov, 2007; Lee et al., 2004; Nunez Miguel et al., 2007; Ozinsky et al., 2000; Saitoh et al., 2004; Zhang et al., 2002). This suggests the SCHOOL-like mechanisms of TLR-4 signaling and imposes the requirement of ligand multivalency. However, the nature of multivalency of lipid A that is widely used in studies of TLR-4 signaling is not clear.

Another example is T cell receptor (TCR), the adaptive immune receptor that recognizes antigenic (agonist) peptides presented by

* Tel.: +1 203 505 3807.
E-mail address: Sigalov@signablok.com

0161-5890/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.molimm.2012.03.031

major histocompatibility complex (pMHC) molecules on the surface of antigen-presenting cells (APCs) and initiates T cell-mediated immune response (Germain, 1994). Ligand binding-driven formation of TCR oligomers is known to result in cell activation (Boniface et al., 1998; Cochran et al., 2000). According to these studies, the receptor dimer represents an "elementary stimulatory unit" formation or rearrangement of which is necessary and sufficient for TCR triggering and initiation of T cell signaling (Boniface et al., 1998; Cochran et al., 2000). Homooligomerization of cytoplasmic domains of TCR signaling subunits has been also reported (Sigalov et al., 2004), suggesting the SCHOOL mechanisms of TCR triggering and signal transduction (Sigalov, 2010a). Taken together, these data strongly support the requirement of ligand multivalency. However, although soluble monomeric agonist pMHCs cannot trigger TCR even at high concentrations (Abastado et al., 1995; Bachmann et al., 1998; Boniface et al., 1998; Cochran et al., 2000; Kiessling et al., 2006; Reich et al., 1997), these molecules were reported to initiate TCR signaling with high sensitivity when surface-bound (Ma et al., 2008).

My hypothesis here is that at certain conditions these as well as many other lipid and protein ligands cannot be considered as truly monovalent. For soluble lipids, ligand valency will critically depend on whether concentrations are above or below a certain threshold concentration, i.e. the critical micelle concentration (CMC) or critical aggregate concentration (CAC). At concentrations above the CMC, lipid ligands form aggregates that are in a dynamic equilibrium with monomers which remain at a constant concentration corresponding to the CMC. For surface-bound molecules, valency of ligands will depend on whether the surface density of these ligands is or is not high enough to provide sufficient receptor proximity to trigger signaling (the critical ligand density, CLD). This hypothesis will be illustrated below by focusing on studies of TLR-4 and TCR signal transduction.

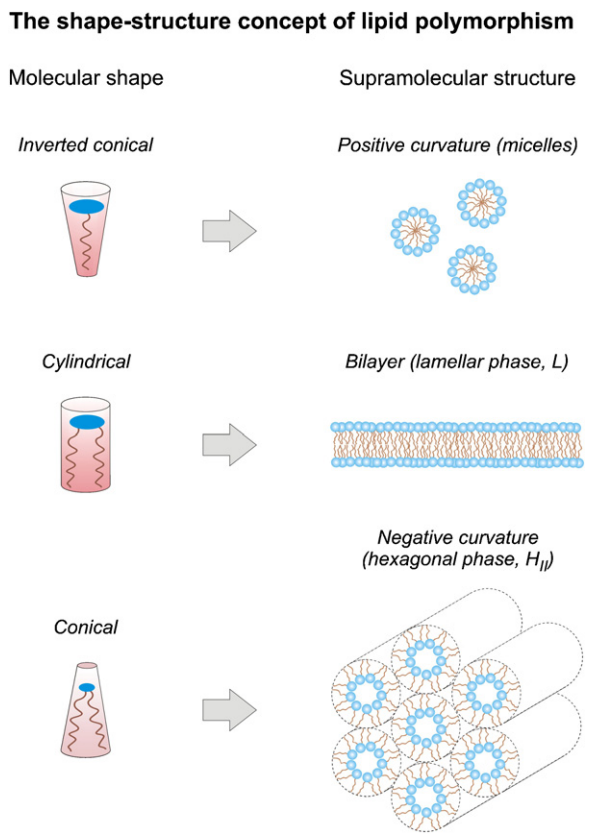

Fig. 1. The shape–structure concept of lipid polymorphism. Molecules with an overall inverted conical shape form supramolecular structures with a positive curvature, such as micelles. Cylindrical-shaped lipid molecules preferentially assemble into lipid bilayers (lamellar phase, L). When lipid molecules have an overall conical shape, aggregate structures with a negative curvature are preferentially formed, such as inverted micelles (not shown) or an inverted hexagonal phase structure (H_{II}).

2. Toll-like receptor-4 signaling

TLR-4 is a key signaling receptor of innate immunity. It recognizes LPS, one of the most immunostimulatory glycolipids, and initiates a proinflammatory signaling cascade. While immunogenicity of LPS is associated with the polysaccharide components, toxicity of LPS is associated with the lipid component (lipid A), the structure of which is highly conserved among Gram-negative bacteria. It should be noted here that despite the common architecture, fine structures of lipid A of different bacterial origin may vary substantially.

Because of an obvious and strong correlation between three-dimensional supramolecular structure and biological activity of various lipid A preparations (Brandenburg et al., 2000; Fukuoka et al., 2001; Schromm et al., 1998; Seydel et al., 1994, 2003), I will start with a brief review of most important biophysical properties of lipids with particular focus on lipid A.

2.1. Lipid polymorphism

Above the CMC (or CAC), amphiphilic lipid molecules form multimeric aggregates in aqueous environment. Below, we will use the term "CMC" to refer to a critical concentration above which supramolecular structures are formed.

Within the shape–structure concept of lipid polymorphism (Cullis and de Kruijff, 1979), molecules that have an overall inverted conical shape form structures with a positive curvature, such as micelles (Fig. 1). Examples are detergent molecules, lysophospholipids and polyphosphoinositides. Lipid molecules with an overall cylindrical shape, such as phosphatidylcholine, when the cross-sectional area of the lipid headgroup is similar to the cross-sectional area of the acyl chains preferentially self-assemble into a liquid crystalline lamellar phase (L), similar to the phase of the cell membrane bilayer (Fig. 1). When lipid molecules have an overall conical shape (Fig. 1), such as diacylglycerol and phosphatidylethanolamine, aggregate structures with a negative curvature are preferred, such as inverted micelles (not shown) or an inverted hexagonal phase structure (H_{II}). Another non-lamellar phase formed by conical-shaped lipid molecules is the cubic phase (Q) with characteristics that are intermediate between an L and H_{II} phases (not shown). It is known that not only the chemical structure of the lipid headgroup and acyl chains but also environmental variables including pH, salt concentration, temperature, presence of divalent cations, and hydration, define the overall shape of the lipid (Cullis and de Kruijff, 1979).

Like other lipids, LPS and free lipid A form supramolecular aggregates above the CMC. Multiple studies have shown that biological activity strongly correlates with the three-dimensional structure of these aggregates (Fig. 2) (Brandenburg et al., 2000; Fukuoka et al., 2001; Schromm et al., 2000, 1998; Seydel et al., 1994, 2003, 2000).

2.2. Lipid A polymorphism and biological activity

The three-dimensional structure of multimeric aggregates formed by LPS and free lipid A in aqueous solutions at concentrations above the CMC depends on the shape of these molecules. In agreement with the shape–structure concept of lipid polymorphism (Fig. 1), cylindrical-shaped molecules of lipid A form a lamellar structures (L), while conical-shaped molecules form