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Computational design principles for bioactive dendrimer based constructs as antagonists of the TLR4-MD-2-LPS complex

Teresa Barata [a,b,1], Ian Teo [a,1], Sanjiv Lalwani [c,1], Eric Simanek [c], Mire Zloh [b], Sunil Shaunak [a,*]

[a] Departments of Medicine, Infectious Diseases & Immunity, Imperial College London at Hammersmith Hospital, Ducane Road, London W12 ONN, UK
[b] Department of Structural Chemistry, London School of Pharmacy, 29 - 39 Brunswick Square, London WC1N 1AX, UK
[c] Department of Chemistry, Texas Christian University, Fort Worth, TX 76129, USA

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ABSTRACT

The cell surface interaction between bacterial lipopolysaccharide (LPS), Toll-like receptor 4 (TLR4) and MD-2 is central to bacterial sepsis syndromes and wound healing. We have shown that a generation (G) 3.5 polyamidoamine (PAMAM) dendrimer that was partially glycosylated with glucosamine inhibits TLR4-MD-2-LPS induced inflammation in a rabbit model of tissue scaring. However, it was a mixture of closely related chemical species because of the polydispersity of the starting PAMAM dendrimer. Generation 2 triazine dendrimers with single chemical entity material status are available at low cost and at the kilogram scale. PAMAM dendrimer can be synthetically grafted onto this triazine core dendrimer to make new triazine-PAMAM hybrid dendrimers. This led us to examine whether molecular modelling methods could be used to identify the key structural design principles for a bioactive lead molecule that could be synthesized and biologically evaluated. We describe our computer aided molecular studies of several dendrimer based constructs and the key design principles identified. Our approach should be more broadly applicable to the biologically focused, rational and accelerated design of molecules for other TLR receptors. They could be useful for treating infectious, inflammatory and malignant diseases.

1. Introduction

Since their introduction in 1985 and subsequent commercial availability, PAMAM dendrimers have served as the major anchoring platform for the exploration of the properties and potential of dendrimers [1]. To this end, PAMAM dendrimers have already been successfully used for diagnostic, sensing, material science and catalysis based applications [2–5].

We have already shown that a partially glycosylated Generation (G) 3.5 PAMAM dendrimer blocked TLR4-MD-2-LPS mediated pro-inflammatory cytokine responses [6]. It inhibited TLR4 mediated inflammation in a rabbit model of tissue scaring (Fig. 1). The biologically relevant cell surface interaction blocked was between bacterial lipopolysaccharide (LPS), host Toll-like receptor 4 (TLR4) and MD-2 [7,8]. Park et al. have recently defined the structural basis of the recognition of LPS by the TLR4-MD-2 complex [9]. In brief, the transport protein CD14 collects and delivers LPS to MD-2. The two phosphorylated glucosamines of the lipid A component of LPS bind to the charged entrance of MD-2′s hydrophobic cavity. The surface residues lining the entrance of MD-2′s pocket that have been shown to have a key role in the electrostatic binding of LPS are Arg90, Lys91, Ser118 and Lys122 [10]. This is followed by the lipid chains of LPS becoming buried in MD-2′s hydrophobic cavity. The activated TLR4-MD-2-LPS complex undergoes conformational changes and receptor dimerization, and this triggers intracellular signaling events [9]. It also initiates the pro-inflammatory chemokine and cytokine cascade responsible for host innate immune responses to pathogens and to surgical tissue injury.

Our recent molecular docking studies have shown that these partially glycosylated dendrimers with a hydrophilic surface bind to the entrance of MD-2′s hydrophobic cavity and prevent the binding of LPS [11]. They form co-operative electrostatic interactions with residues lining the entrance to MD-2′s hydrophobic pocket (Fig. 2). Crucially, dendrimer glucosamine interferes with the electrostatic binding of: (i) the 4′phosphate on the di-glucosamine of LPS to Ser118 on MD-2; (ii) LPS to Lys91 on MD-2; (iii) the subsequent binding of TLR4 to Tyr102 on MD-2. This is followed by additional co-operative interactions between several of the dendrimer glucosamine's carboxylic acid branches and MD-2. Collectively, these interactions block the entry of the lipid chains

* Corresponding author. Tel.: +44 20 8383 2301.
E-mail address: s.shaunak@imperial.ac.uk (S. Shaunak).
[1] These authors contributed equally to the work.

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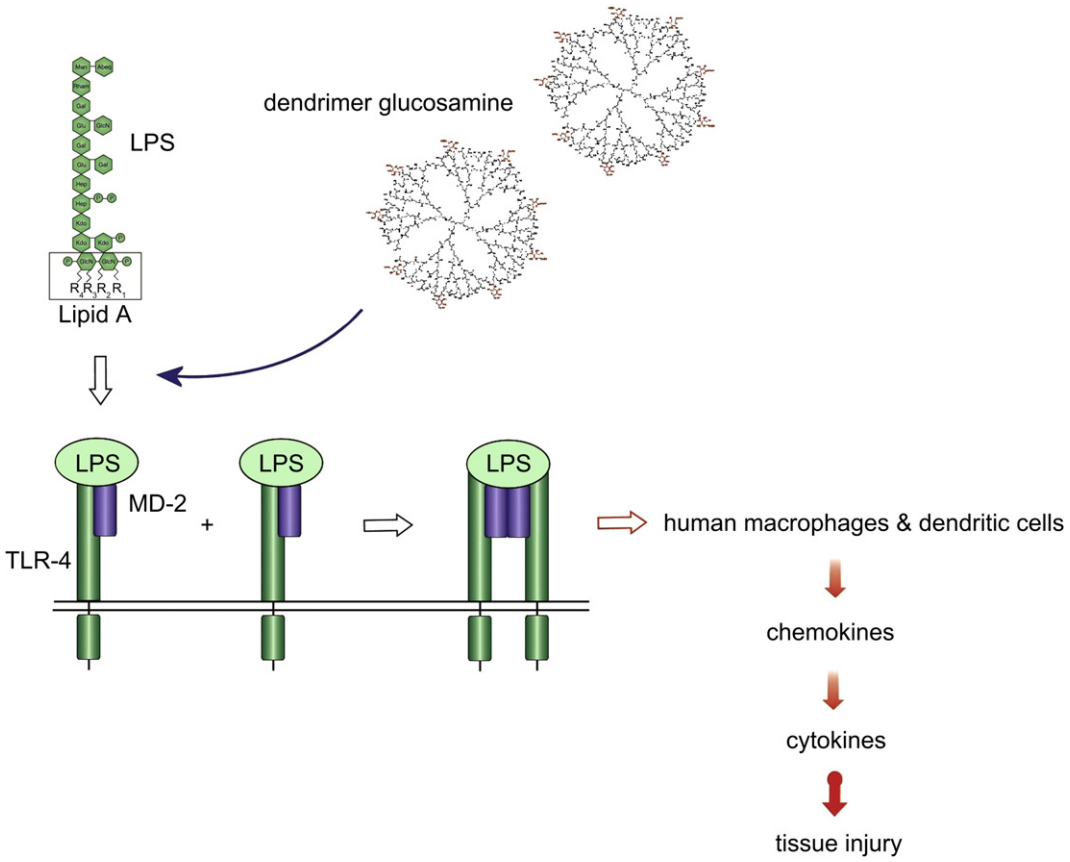

Fig. 1. Illustration of the competition between LPS (agonist) and the partially glycosylated dendrimer (antagonist) for TLR4-MD-2-LPS complex induced pro-inflammatory cytokine production.

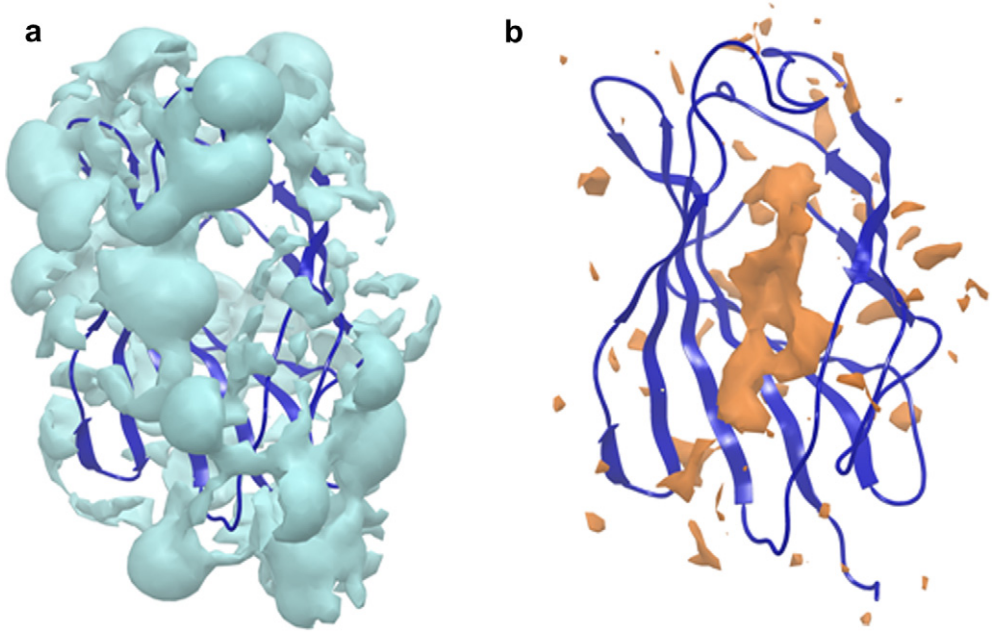

Fig. 2. a and b Frontal view of the hydrophilic and hydrophobic surfaces of MD-2: The hydrophilic (cyan) surface of MD-2 defines the need for any new dendrimer based construct to also have a hydrophilic surface. In contrast, the hydrophobic (orange) area, to which the acyl chains of Lipid A bind, lies buried deep inside MD-2′s cavity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)