Targeting intracellular mediators of pattern-recognition receptor signalling to adjuvant vaccination

J. Wales, E. Andreakos, M. Feldmann and B. Foxwell1
Kennedy Institute of Rheumatology, Imperial College London, 1 Aspenlea Road, London W6 8JH, U.K.

Abstract
PRR (pattern-recognition receptor) signalling is involved early in the immune response and therefore would be attractive to target during vaccination. The use of PRR ligands has shown some success; however, toxicity and non-specificity are issues with this strategy. The targeting of PRR intracellular signalling networks would allow for greater specificity and reduced systemic toxicity. The present review examines the successes seen with overexpression or repression of PRR signalling molecules.

Introduction
Since their discovery over 10 years ago, the PRRs (pattern-recognition receptors) have been shown to be crucial in innate immunity. They consist of extracellular TLRs (Toll-like receptors), as well as intracellular receptors [some TLRs, Nod (nuclear oligomerization domain) etc.]. The PRRs are able to recognize microbial components, known as PAMPs (pathogen-associated molecular patterns). This pathway is involved early on in the immune response and would be a good target to manipulate during vaccination. Vaccination is effective at controlling infectious disease; however, we now live in an environment where a high quality of vaccination is expected, but the side effects are unwanted. Therefore new vaccination strategies must be evaluated. The evidence for the TLR pathways being a good target during vaccination comes from the use of FCA (Freund’s complete adjuvant). FCA is made from an oil emulsion that contains homogenized mycobacteria. It is well documented that this adjuvant is able to induce strong immune responses to protein antigen and it is likely that the PAMPs present in the mycobacteria are largely responsible for this response; however, this has not been definitively proven. As FCA is not licensed for use in humans, more targeted strategies, such as the use of individual PRR ligands, must be examined.

TLR ligands have been shown to be effective vaccine adjuvants. Vaccines for HIV have contained ligands for TLR4, TLR2 and 6, TLR7 and 8, and TLR9 and have been shown to enhance specific antibody responses [1,2]. However, there are drawbacks with this technique. First, if ligands are administered systemically, their inherent toxicity may cause aberrant responses and exacerbate disease states in vaccines. For example, lipopolysaccharide is involved in neurodegenerative disorders [3]. Secondly, the TLR ligand must be present in the extracellular milieu to act upon its cell-surface receptor. Once there, the ligand will not necessarily act upon the same cell as the antigen but on other cells in the vicinity as well.

The targeting of intracellular signalling networks, rather than extracellular ones, would alleviate these problems. If a DNA or viral-based expression system were used, the molecular adjuvant would not have to be exported from the vaccine-transfected cell. Therefore (i) the adjuvant would target the same cell as the antigen, allowing greater specificity. (ii) As the adjuvant stays in the cell, if too much adjuvant is produced, it would not cause a systemic but rather a unicellular toxicity, which is arguably advantageous to the immune response. (iii) The adjuvant would reach threshold levels with faster kinetics due to its confinement within the cell, rather than being diluted in the extracellular milieu.

The targeting of intracellular PRR networks would allow a more specific and less toxic response.

PPR signalling
The signalling networks induced by PRRs are complex and some of the key adaptors are discussed below. The principal adaptor for TLR signalling is MyD88 (myeloid differentiation factor 88) as it was shown to be involved in all TLR signalling except for TLR3. The TIR [Toll/IL (interleukin)-1 receptor] domain of TLRs recruits the adaptor proteins to mediate the activation of various transcription factors, such as NF-κB (nuclear factor κB) and members of the IRF (interferon regulatory factor) family. Other signalling molecules include Mal (MyD88-adaptor-like) (TLR2 and 4), TRIF [TRIF domain-containing adaptor protein inducing IFN-β (interferon β)] (TLR3 and 4) and TRAM (TRIF-related adaptor molecule) (TLR4). Another signalling
After PRR activation by their ligands, intracellular signalling occurs. The principal mediator of TLR signalling is MyD88, with Mal, TRIF and TRAM also being involved. After activation of Nod1/2, signalling occurs through RIP2 (receptor-interacting protein 2) and NIK to activate NF-κB. NIK is also involved in TLR2 signalling. The transcription factors activated as a result of these signalling cascades include NF-κB and IRFs. SOCS1 down-regulates signalling through TLR4 by mediating the degradation of Mal. These pathways are attractive to target during vaccination and the molecules that have been examined as adjuvants are shown in grey.

Another signalling molecule, NIK, was shown to be an effective vaccine adjuvant. Our studies have shown that NIK overexpression can induce an NF-κB reporter gene without the presence of upstream stimuli [8]. When overexpressed in dendritic cells, NIK was able to induce a mature phenotype with increased cytokine production (TNF-α, IL-12, IL-15 and IL-18) and the presence of cell-surface markers (MHC I/II and co-stimulatory molecules) [9]. Adenoviruses were used to express both a reporter antigen and NIK. NIK was able to increase the antibody response above that of antigen alone and directed the antibody profile towards IgG2a. NIK also induced a strong IFN-γ and CTL response above that of antigen alone, indicating the efficient activation of cell-mediated responses [9].

Overexpression of transcription factors themselves has also been examined. Sasaki et al. [10] overexpressed IRF-1, -3 and -7 in a DNA vaccine. IRF-1 induced strong antibody immune responses, whereas IRF-3 and -7 induced strong cellular immune responses. The cellular responses were shown to be a mixture of IFN-γ- and IL-4-producing T-cells.

The down-regulation of intracellular signalling repressors has also been examined. Several studies by Chen and colleagues have evaluated the knock-down of SOCS1 using siRNA (small interfering RNA) as a molecular adjuvant [11, 12]. Initially they showed that the presence of this siRNA caused dendritic cells to be more responsive to cytokines and TLR ligands. Dendritic cells treated with the siRNA and pulsed with antigen ex vivo were introduced into the host as a vaccine. These treated cells were able to induce strong IFN-γ responses.
and CTL responses, which were able to clear a tumour challenge [11]. When HIV antigens were used to pulse these cells, a strong IgG2a antibody response was seen where the immune response lasted greater than 6 months [12].

**Summary**

The PRR pathways are an attractive source of molecular adjuvants for vaccines due to their involvement early in the immune response. Intracellular signalling molecules are effective molecular adjuvants due to their ability to stay confined in the producing cell, allowing a greater, more specific and less toxic immune response. The overexpression of MyD88, TRIF, NIK and IRFs, as well as the down-regulation of SOCS1, have all been shown to be effective molecular adjuvants. This field is in its infancy and as the PRR signalling pathways become more defined, more candidates for molecular adjuvants may appear. This class of adjuvants induce strong cell-mediated immunity along with antibody responses, showing their promise for inclusion in future clinical vaccine preparations.

**References**


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