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Review

Natural products and the search for novel vaccine adjuvants

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ABSTRACT

Vaccines that protect against intracellular infections such as malaria, *Leishmania* and *Chlamydia* require strong cellular responses based on $CD4^+$ T cells and $CD8^+$ T cells in addition to antibodies. Such cell-mediated responses can be potentiated with adjuvants. However, very few adjuvants have been licensed for use in humans; thus there is an urgent need for the discovery of new non-toxic adjuvants in order to produce more efficacious vaccines. Until recently, the mechanisms of how adjuvants worked remained largely unknown, but, it is becoming clearer that many function via host germline-encoded pattern recognition receptors (PRRs) expressed by most immune and non-immune cells. Most PRRs sense infection and transmit a series of signals that ultimately lead to the development of immunity. PRR mediated signalling can be harnessed to search for new vaccine adjuvants. Dendritic cells (DCs) express many PRRs and are remarkably effective at directing T cell immunity. Natural products (NPs) have been the basis of many drugs and are a rich source of immune activators and/or regulators of the immune response. Here we review PRRs in the context of NPs and propose the use of DCs as biological probes to help identify novel immune type molecules and adjuvants within collections of NPs.

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Abbreviations: NPs, natural products; MDP, muramyl dipeptide; PGN, peptidoglycan; NOD1, nucleotide-binding oligomerization domain 1; CLRs, C-type lectin receptors; NLRs, nucleotide oligomerization domain-like receptors; RLRs, RIF-I-(retinoic acid-inducible gene 1)-like receptors; NLP3, NACHT-LRR-PYD-containing protein 3; CRD, carbohydrate recognition domains; FLS2, flagellin-sensitive receptor; SLIP, LPS-interacting protein; NB-LRR, nucleotide binding site and leucine rich repeat receptors; TIR, Toll-interleukin-1 receptor; TIR-NB-LRR, NB-LRR proteins containing TIR; EGCG, epigallocatechin-3-gallate; GalCer, alpha-galactosylceramide; NPLs, natural product libraries; HTS, high throughput screening; MoDCs, human monocyte-derived dendritic clls; iDCs, immature dendritic cells; pDCs, plamacytoid dendritic cells.

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1. Introduction

Adjuvants are substances that increase or modulate the immunogenicity of an antigen while at the same time lower the concentration of antigen required for vaccination [1,2]. As compared with whole-cell or virus-based vaccines, subunit vaccines are poorly immunogenic and require the presence of adjuvants to stimulate protective immunity [1,3]. However, currently licensed adjuvants such as alum predominantly induce antibody responses [4]. Adjuvants that help induce T cell-mediated immunity would facilitate the design of new vaccines for infectious diseases and cancer [5]. A major advance in the understanding of how vaccine adjuvants function is the discovery that signals derived from pattern recognition receptors (PRRs) can activate dendritic cells (DCs) so that efficient priming and activation of T cells can be achieved. The best example is the Toll-like receptors (TLRs) family of PRRs whose ligation by microbial signatures leads almost invariably to T cell-mediated immunity in which DCs play a major role [6].

PRRs or analogues of PRRs are expressed by most living organisms across species from plants, sponges to vertebrates [7]. In mammals, signals delivered from PRRs influence antigen presenting cells, such as DCs, with respect to the strength and type of immunity that is developed against bacterial, viral or fungal infections [8]. In invertebrates, PRRs also function to provide protection against a variety of infectious agents. Because of the high levels of conservation among PRRs across species, ligands activating PRRs in lower organisms may also activate PRRs in mammals where they could be employed to modulate immune function. However, the natural ligands for PRRs expressed by plants, marine organisms and other biota remain undefined.

Natural products (NPs) are a primary source of many pharmaceuticals used today [9–11] and are a potential source of immune modulating compounds. The discovery of such compounds could result in the development of new adjuvants for vaccines and drugs for the treatment of other diseases such as allergy and cancer where immune modulating therapies are greatly needed. It is envisioned that new functional screening procedures and the availability of pre-fractionated libraries of NPs will facilitate the identification of such molecules. This review briefly summarizes the current body of knowledge of PRRs in the context of immunity in vertebrates and lower organisms, and proposes a new conceptual procedure to identify potential immune modulating compounds within collections of NPs.

2. PRRs and the triggering of immunity

For many years, the gold standard adjuvant has been complete Freud's adjuvant (CFA), an emulsion of killed mycobacteria in oil [12]. More recently, different bacteria including *Mycobacterum* spp, *C. parvum*, *B. pertusis*, *N. meningitidis* in full or as bacterial products have been employed as vaccine adjuvants. It is clear that most adjuvant effects of CFA and these bacteria are mediated though PRRs [13]. Indeed, PRRs are host sensors that play a leading role in the activation of the immune response in vertebrates [14]. PRRs interact with highly conserved microbial structures known as pathogen associated molecular patterns (PAMPs). Following microbial recognition, PRRs transmit signals that trigger innate immunity enabling phagocytic cells, such as macrophages and neutrophils, to mediate pathogen uptake and killing [15]. This ultimately results in the initiation of proinflammatory responses and induction of adaptive immunity through the activation of DC [16,17]. Not all PRRs are equal in terms of their ability to trigger immunity. While some PRRs such as TLRs are able to induce both B and T cell responses, other PRRs such as macrophage mannose receptor (MMR) are not competent enough to induce an adaptive immune response on their own [8]. Major families of PRRs contributing to the development of immunity in mammals and their defence role in invertebrates and plants are briefly discussed below.

2.1. Nucleotide oligomerization domain-like receptors (NLRs)

NLRs are cytosolic molecules that sense the presence of intracellular PAMPs [18,19]. Muramyl dipeptide (MDP) is a derivative of intracellular bacterial peptidoglycan (PGN) and is recognized by nucleotide-binding oligomerization domain 1 and 2 (NOD1 and NOD2), a member of the NLRs. Stimulation of NOD molecules by MDP drives antigen-specific immunity into a predominant Th2 polarization profile. Nevertheless, stimulation of NOD1 together with TLRs (see below) also triggers Th1 or Th2 or Th17 activation pathways [20] demonstrating that these NLRs potentiate responses depending on signals derived from co-engaged receptors. Another member of the NLR family is the NACHT-LRR-PYD-containing protein 3 (NLP3), a component of the inflammasome—a high molecular weight complex that functions as an intracellular sensor of 'danger' [21]. Activation of NLP3 by alum triggers the activation of inflammatory DCs and subsequent development of Th2 responses [22,23]. However, the absence of NLRP3 does not seem to significantly impact T cell and B cell responses in mice following immunization with alum [24]. Thus, the exact role played by NLRP3 for alum adjuvanticity remains undefined. Shellfish shell-derived chitosan and the saponin Quil-A are also known adjuvants that activate NLP3 signalling [25]. In general, derivatives of NLP3 strongly correlate with Th2 driven antibody responses [14].

2.2. C-type lectin receptors (CLRs)

CLRs comprise a large group of heterogeneous PRRs found almost exclusively in the Metazoa [7,26,27]. CLRs were originally defined as calcium-dependent carbohydrate-binding proteins [1,27]. CLRs also include proteins without calcium binding or carbohydrate specificity and, therefore, CLRs are now defined as proteins that contain C-type lectin domains – carbohydrate recognition domains (CRD) of mannose-binding lectin [27]. CLRs contain at least one CRD of ~130 amino acid residues in length and are highly conserved in vertebrates [28]. CLRs have quite diverse immune function. While some are involved in recognition of self and develop functions of immune homeostasis others are true PRRs implicated in host immune response [27,29,30]. For example, the mannose-binding lectin (MBL) is a serum protein that activates complement and provides defence against various bacterial infections including *Staphylococcus aureus* and *Streptococcus pneumoniae* [31]. Another CLR, langerin, expressed by langerhan