



Innate sensors that regulate vaccine responses

Philipp Georg and Leif E Sander

Pattern recognition receptors (PRRs) control elemental functions of antigen presenting cells (APCs) and critically shape adaptive immune responses. Wielding a natural adjuvanticity, live attenuated vaccines elicit exceptionally efficient and durable immunity. Commonly used vaccine adjuvants target individual PRRs or bolster the immunogenicity of vaccines via indirect mechanisms of inflammation. Here, we review the impact of innate sensors on immune responses to live attenuated vaccines and commonly used vaccine adjuvants, with a focus on human vaccine responses. We discuss the unique potential of microbial nucleic acids and their corresponding sensing receptors to mimic live attenuated vaccines and promote protective immunity.

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Department of Infectious Diseases and Pulmonary Medicine, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

Corresponding author: Sander, Leif E (leif-erik.sander@charite.de)

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Introduction

According to estimates of the World Health Organization (WHO), vaccines save approximately 2.5 million lives per year [1], which arguably makes them one of the most effective public health interventions of all times. The declaration of ‘vaccine hesitancy’ as one of the top-ten threats to global health by the WHO [2], further underscores the extraordinary importance of vaccines for human health and prosperity. Live vaccines, such as the measles vaccine or the yellow fever vaccine, provide lifelong immunity in the majority of vaccinees. Residual safety risks, albeit minor, limitations to use of live vaccines under certain conditions such as immunosuppression or pregnancy, and a relatively demanding production process, created a need for safer and cheaper alternatives. Subunit vaccines, first introduced a century ago by Behring [3] and Ramon [4], greatly lowered

production costs and the risk of unwanted side effects, yet at the expense of decreased immunogenicity and efficacy. Non-live vaccines generally require additional immunostimulatory agents, called adjuvants, in order to elicit protective immunity. Described in 1926 by Alexander Glennie to critically enhance the immunogenicity of diphtheria toxoid, aluminum potassium sulphate and other aluminum salts, commonly known as alum, are the most widely used vaccine adjuvants [5], but their modes of action still remain incompletely understood [6]. Dendritic cells (DCs), famously referred to as ‘nature’s adjuvant’ by Ralph Steinman [7,8], and other antigen presenting cells (APCs) integrate external cues like microbial stimuli, and process them into signals that facilitate T cell priming [9–11]. Innate immune signals and pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) control fundamental processes in APCs, including antigen uptake and presentation, DC maturation and migration, production of cytokines, and antimicrobial activity [12–14]. PRR engagement, therefore, critically shapes the magnitude and the quality of adaptive immune responses to vaccine antigens [15–18], and their discovery two decades ago spurred efforts to target PRRs for vaccine purposes [19]. Agonists of TLR4 and TLR9 have since been proven to be safe and effective adjuvants that are now included in selected commercial vaccines [20–22]. Several other PRR ligands are currently under investigation as vaccine adjuvant candidates or immunotherapeutics [23–25].

Here, we summarize existing evidence on the function of innate immune sensors in shaping vaccine responses, with a specific focus on available data from human studies, or closely related experimental models.

A nut for every bolt — distinct roles of PRRs in T cell immunity and vaccine responses

Rather than binary on–off switches of APC maturation, PRRs relay precise information about the nature and the threat level of microbial encounters to the immune system [26]. By controlling key functions of APCs, differential PRR engagement by distinct classes of microorganisms confers specificity to the immune response by eliciting diverse cellular programs and imprinting subsequent effector T cell responses [12–14,27].

Yet, with few exceptions, the contributions of individual PRRs to human T helper cell-response and vaccine responses are not well defined. The following sections summarize the available evidence on the role of selected PRRs and their various ligands on adaptive immunity,

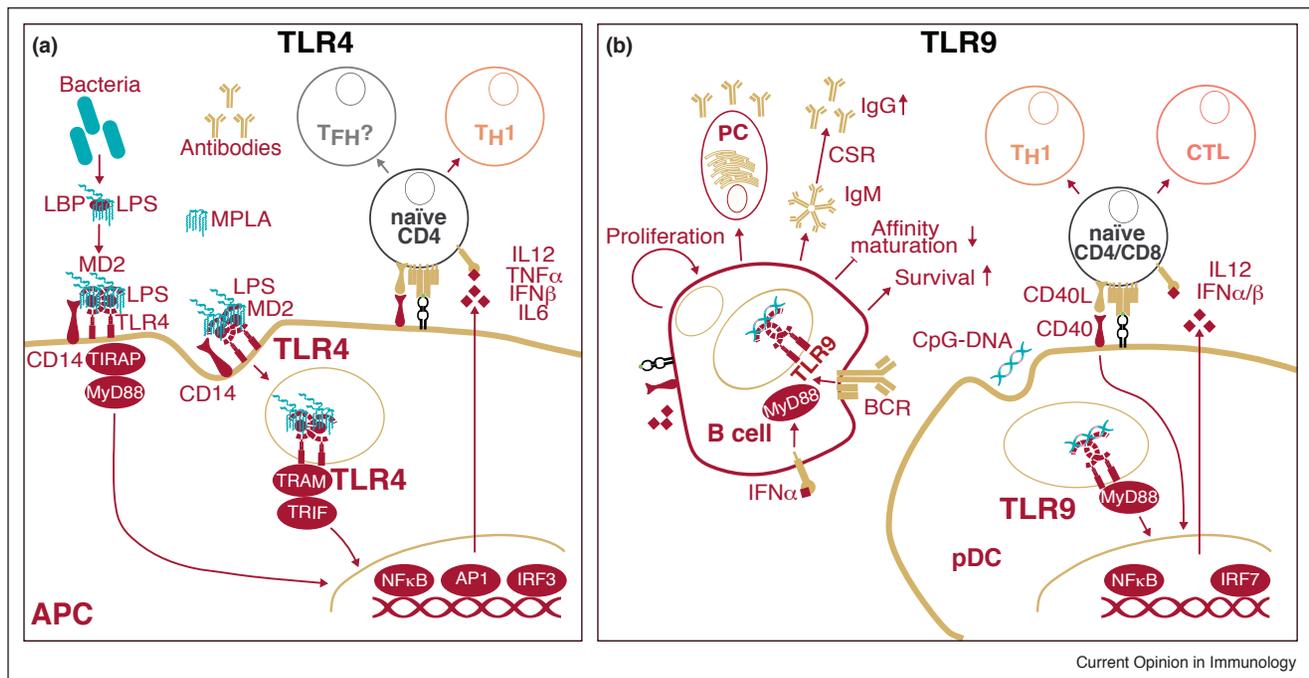
with a focus on those PRR ligands with the most advanced (pre)clinical stages of development.

Bacterial lipopolysaccharide (LPS) has been known for its adjuvant effects and its ability to augmented antibody responses to protein antigens since the 1950s [28]. Yet, the molecular basis for its adjuvanticity remained obscure until the discovery of TLR4 and its role in APC activation, roughly half a century later [29,30] (Figure 1a). Monophosphoryl lipid A (MPLA), a detoxified LPS derivative, which stimulates ‘TIR-domain-containing adaptor inducing interferon- β ’ (TRIF)-biased TLR4 signaling [31], was the first TLR agonist to be approved as a vaccine adjuvant for clinical use in humans [20,32]. MPLA-containing adjuvants are currently used in a human papilloma virus (HPV) vaccine and the malaria vaccine candidate RTS,S [20,33]. LPS, MPLA, and synthetic TLR4 ligands such as GLA-SE generally promote T_H1 -biased immune responses [32,34,35] (Figure 1a). Co-encapsulation of MPLA and TLR7 ligand imiquimod (R837) or TLR7/8 agonist resiquimod (R848) into nanoparticles further enhances the magnitude and longevity of

antibody responses to protein antigens in mice and non-human primates [15]. Murine B cells respond to LPS with a robustly increased proliferation and antibody production; in contrast, human B cells are largely unresponsive to TLR4 stimulation [36]. While TLR4 was long believed to be the sole mammalian LPS receptor, it was recently revealed that cytosolically delivered LPS triggers non-canonical inflammasome activation via Caspase-11 in mice, and Caspase-4 in humans [37,38]. The contribution of Caspase-4/11 and non-canonical inflammasomes to vaccine responses is currently unknown.

Intracellular nucleic acid sensors detect invading microorganisms, particularly viruses. Given the efficient induction of long-lived immunity by many viral infections and live attenuated vaccines (LAVs), nucleic acid sensors have long been viewed as ideal targets for vaccine adjuvants [39]. Endosomal TLRs (TLR3, TLR7, TLR8 and TLR9) sense different types of nucleic acids, or degradation products thereof. Their highly cell type-specific expression pattern in human immune cells contributes to their diverse effects on adaptive immune responses [40].

Figure 1



TLR4 and TLR9 adjuvant sensing and the impact on adaptive immune responses.

(a) LPS and derivatives like MPLA are sensed by TLR4 together with MD2 and CD14. LPS recognition is enhanced by accessory lipopolysaccharide binding protein (LBP). TLR4 signals via adaptor molecules TIRAP-MyD88, or via TRAM-TRIF upon CD14-mediated endocytosis. TLR4 signalling leads to activation and translocation of NF- κ B, AP1, and IRF3 and subsequent production of pro-inflammatory cytokines and type I IFN, and upregulation of co-stimulatory molecules. TLR4 activation promotes predominantly T_H1 responses and antibody production. **(b)** TLR9 is primarily expressed by B cells and pDCs. It senses hypomethylated CpG DNA sequences upon endosomal internalization. TLR9 activation in pDCs leads to upregulation of co-stimulatory molecules and potent induction of type I IFN via IRF7. Combined stimulation via TLR9 and CD40L induces production of IL12p70. TLR9 activation induces predominantly T_H1 and cytotoxic T lymphocyte (CTL) responses. TLR9 activation in B cells increases proliferation, survival and plasma cell (PC) differentiation. TLR9 enhances antibody production and class switch recombination (CSR).

TLR9 senses hypomethylated cytosine-phosphate-guanine (CpG) motifs abundantly present in prokaryotic DNA [41,42]. Ligation of TLR9 by bacterial DNA or CpG oligodesoxynucleotides (CpG-ODN) induces DC maturation, type I IFN production, and IL-12 production in plasmacytoid DCs (pDCs) [41,43] (Figure 1b). TLR9-induced IL-12p70 production by pDCs requires CD40-CD40L co-stimulation. Activation of TLR9 enhances robust type 1 immunity in humans and mice [44–46], which has prompted significant efforts to develop TLR9 agonists as immunotherapeutics for cancer [46,23]. Overall, CpG ODNs showed a good safety profile, but therapeutic effects were rather modest or insignificant in the majority of clinical trials [47,48]. In contrast to the disappointing results as an immunotherapeutic agent in cancer, CpG-ODN 1018 was recently approved by the FDA as an adjuvant in a novel hepatitis B virus (HBV) vaccine [49]. The new CpG-adjuvanted vaccine requires only two injections and showed increased immunogenicity compared to the three-dose regimens of existing HBV vaccines [22]. TLR9 is highly expressed in human B cells, where it enhances cell survival, plasma cell differentiation, and IgG production [50,51] (Figure 1b), and this effect has been leveraged to generate human monoclonal antibodies *in vitro* [52]. In contrast, it was recently reported that B cell-intrinsic TLR9 signaling inhibited antigen capture and presentation, thereby, limiting T cell help and affinity maturation. The authors suggested that TLR9 stimulation increases the magnitude of antibody responses, but fails to increase antibody affinity maturation [53]. However, as other studies have reported increased high-affinity antibodies in response to CpG-adjuvanted vaccines [18,54], this aspect will require further investigation.

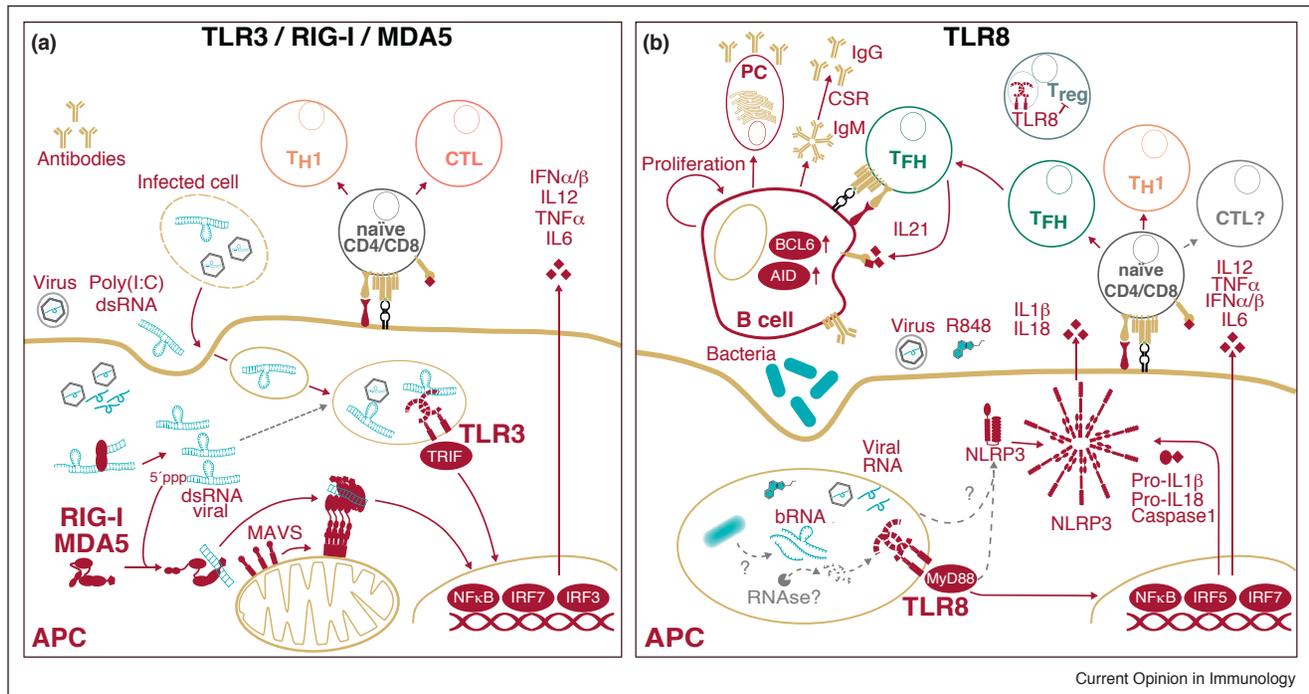
Cytosolic DNA stimulates immune responses via cyclic-di-AMP-GMP (cGAMP) synthetase (cGAS). Acting as a second messenger, cGAMP activates stimulator of IFN genes (STING) leading to IRF3- and NF- κ B activation and DC maturation [55]. The cGAS-STING pathway has a central role in host defense against a variety of intracellular pathogens [56,57], whereas aberrant activation can promote autoimmunity [58–60]. STING is also a critical sensor for bacterially derived cyclic-di-nucleotides [61]. Mounting evidence from preclinical mouse models suggests that cGAS-STING activation promotes type I immunity, which has sparked intense efforts to harness STING agonists for cancer immunotherapy [62,63]. While STING activators have also shown promising results as vaccine adjuvants in mouse models, their role in human T cell-responses and vaccine responses remains to be investigated. An interesting study recently reported an unexpected antiproliferative function of STING in human T cells [64]. Patients harboring activating mutations in *TMEM173* encoding STING display decreased T cell proliferation and lower numbers of memory T cells [64]. While this function of STING could help to control

retroviral infections, its potential impact on STING-targeted vaccine strategies needs to be considered.

Microbial RNA and RNA mimics are well-known immunostimulants. Double-stranded RNA (dsRNA) and synthetic dsRNA analog polyinosinic:polycytidylic acid (poly(I:C)) target the endosomal dsRNA sensor TLR3 [65], expressed primarily in conventional DC and macrophages [40,65]. Cytosolic poly(I:C) and 5'-triphosphorylated RNA (5'-pppRNA), especially viral RNAs, are sensed by DExH/D-box RNA helicases retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), which are ubiquitously expressed [66]. Activation of TLR3 and RIG-I/MDA5 triggers robust type I interferon (IFN) production and anti-viral host responses (Figure 2a). Originally developed as an antiviral agent in the 1960s, poly(I:C) has also attracted significant attention for its adjuvant activities [67]. Poly(I:C) adjuvantation enhances CD4 T cell responses and antibody production to protein vaccine antigens in non-human primates [68]. Subcutaneous application of poly(I:C) elicits innate immune responses with significant similarities to the transcriptional profiles induced by the yellow fever vaccine (YF-17D) in healthy human volunteers [69]. However, the clinical use of poly(I:C) has been limited, in parts by frequent side effects like erythema, fever, headache, arthralgia, and in some cases life-threatening shock, likely due to strong IFN induction [67,70]. Coformulation with carboxymethylcellulose and poly-L-lysine as stabilizing agents (poly(ICLC)) alleviates some of the side effects, and preserves beneficial effects such as antibody production and CD4 T cell expansion, as demonstrated in early phase human cancer-vaccine trials [23,25,71]. Induction of type I IFN is critical for poly(I:C)-mediated adjuvanticity in mouse models [72]. The role of IFN signals in human vaccine responses is less clear [72,73]. While IFN α enhances T_{H1} functions in human memory T cells, it fails to promote T_{H1} differentiation from naïve T cells [74], whereas IFN β antagonizes some T_{H1}-driven pathologies [75]. T follicular helper (T_{FH}) cells are crucial providers of B cell help, required for the formation of high-affinity antibodies and memory B cells [76,77]. Whereas RIG-I-like receptors and type I IFNs have been implicated in T_{FH} cell differentiation in mice [78,79], their contribution to human T_{FH} cell responses remains unclear [80–82]. Current efforts focus primarily on the development of poly(ICLC) and related agents for therapeutic cancer vaccines.

The endosomal ssRNA sensors TLR7 and TLR8 surveil phagocytic cargo for the presence of microbial ssRNA [83,84]. The ectodomains of TLR7 and TLR8 contain two distinct ligand interaction sites that facilitate the recognition of RNA degradation products and small synthetic nucleoside analogs, including imiquimod/R837 (TLR7) and resiquimod/R848 (TLR7/8) [85–87]. Murine

Figure 2



Sensing of microbial RNA and common RNA- and nucleoside analogs and the effects on adaptive immune responses.

(a) TLR3 senses dsRNA, for example in virus infected cells, and synthetic dsRNA analog poly(I:C). TLR3-TRIF signalling induces production of IFN β and inflammatory cytokines, and upregulation of co-stimulatory molecules. The cytoplasmic RNA helicases RIG-I and MDA5 sense primarily viral dsRNA and poly(I:C). RIG-I senses 5'-pppRNA or short poly(I:C) molecules, while MDA5 recognizes long dsRNA or poly(I:C). Ligand binding leads to oligomerization and formation of MAVS filaments and subsequent activation of IRF3/IRF7-dependent IFN α/β production. TLR3/MDA5/RIG-I activation promotes T_H1 and CTL responses and enhances antibody production. **(b)** TLR8 senses degradation products of microbial ssRNA and synthetic nucleosides like R848. Activation of TLR8 induces the production of IL-12p70 and other inflammatory cytokines, as well as IFN α/β . TLR8 agonists also induce activation of the NLRP3 inflammasome through an unknown mechanism. TLR8 stimulated APCs promote T_{FH} differentiation and subsequent GC responses, as well as T_H1 and CTL responses. Treg-intrinsic TLR8 signalling inhibits their regulatory function. Pathways whose role is not fully characterized are depicted in grey symbols and grey dotted lines.

and human TLR7 share ligand specificities and signaling pathways, however, human TLR7 expression is mainly restricted to pDC and B cells [40]. In contrast, TLR8 is broadly expressed in both species, but murine TLR8 shows a defective response to ssRNA [40,83]. The distinct expression patterns contribute to the functional differences of TLR7 and TLR8 and their effects on adaptive immunity. Both TLR7 and TLR8 signal through MyD88 and, depending on the cell type, activate NF- κ B and AP-1, and/or IRF7 with consecutive production of inflammatory cytokines and type I IFN (Figure 2b). Several TLR7 and TLR7/8 ligands have been tested in clinical and pre-clinical trials as immunotherapeutics and vaccine adjuvants [67,88,89]. Topical application of TLR7 ligand imiquimod is a well-established treatment for HPV infection (genital warts), superficial basal cell carcinoma, and actinic keratosis [89]. Imiquimod has also been used as an adjuvant in an experimental JC-virus (JCV) vaccine to treat progressive multifocal leukoencephalopathy (PML) [90,91]. PML is a devastating condition caused by JCV infection of the

central nervous system in immunocompromised hosts. A small number of patients with PML and non-HIV related CD4 lymphopenia received the JCV VP1 protein vaccine adjuvanted with topical imiquimod at the injection site. All patients developed VP1-specific CD4 T cells, cleared JCV, and showed unprecedented neurological improvement [90,91].

The dual TLR7/8 agonist resiquimod (R848) also exerts antiviral activity and promotes antigen-specific T_H1 and CD8 T cell responses [16,92]. However, similar to many other TLR7 and TLR8 agonists, resiquimod is limited by a narrow therapeutic window when given systemically, due to side effects like fever and headache [93]. Poor tolerability profiles may be substantially improved by altered adjuvant formulation [94].

Durable high-affinity antibody responses require highly orchestrated interactions between B cells and cognate T_{FH} cells [76,77]. Targeted mobilization of antigen-specific T_{FH} cell responses would therefore be highly

desirable for vaccine purposes. The complex development of T_{FH} cells and their molecular interactions with B cells have therefore been subject of intense investigation [76,77,95]. In contrast, the role of APC-derived signals in early stages of T_{FH} cell differentiation, especially in humans, remain less well defined. Selective activation of TLR8 in human APC was recently identified as a potent driver of T_{FH} cell differentiation and enhanced vaccine responses [81] (Figure 2b), which will be discussed in greater detail below.

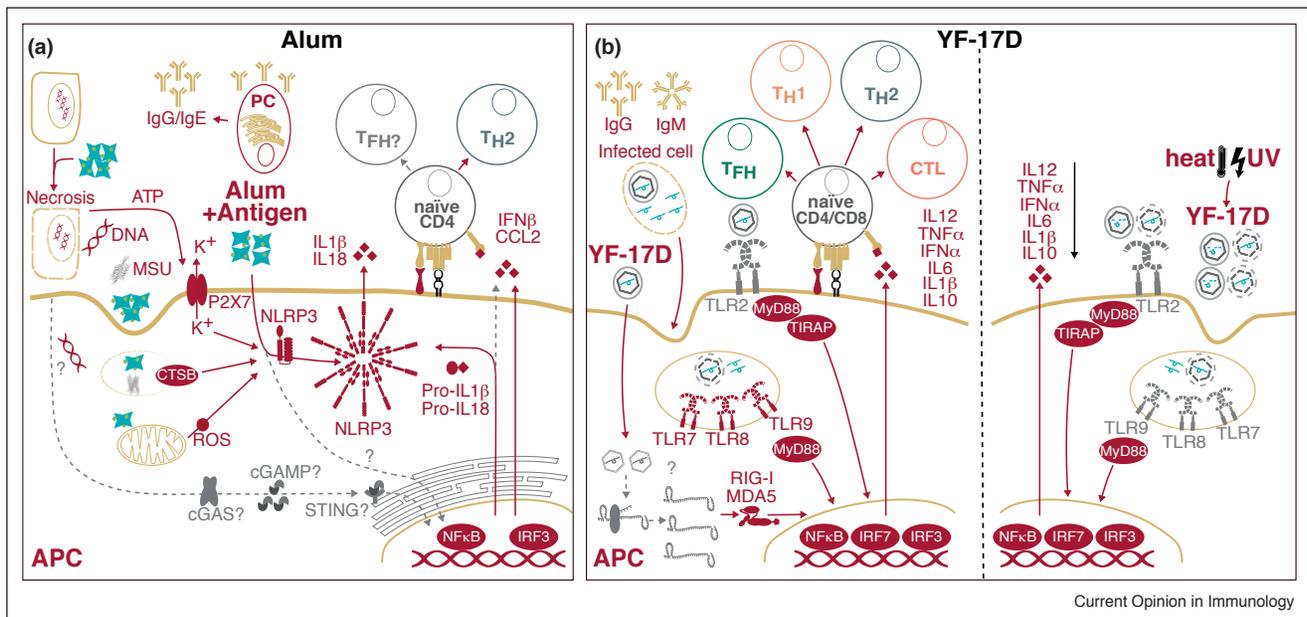
Interestingly, TLR7 and TLR8 are also expressed in human T cells. Here, TLR7 signals may promote T cell anergy [96], while TLR8 activation in human T regulatory cells (Treg) limits their suppressive function through inhibition of glucose metabolism [97,98] (Figure 2b). The effects of lymphocyte intrinsic TLR7 and TLR8 signals will require further investigation, but they should be considered when applying unencapsulated nucleoside analogs as vaccine adjuvants and immunotherapeutics.

The well-hidden secrets of Alum

The most widely used vaccine adjuvants, such as alum and oil-in-water emulsions, do not directly engage PRRs. In fact, their exact modes of action still remain largely

obscure. Aluminum salts have been used as vaccine adjuvants since the 1930s [5,6]. For decades, alum was thought to exert its adjuvant activity mainly by retarding antigen release and prolonging antigenic exposure. However, more recent re-evaluations have called this ‘depot theory’ into question. Within hours upon injection, alum causes pronounced local inflammation, including the release of IL-1 β , CCL2, histamine, and IL-5, with subsequent recruitment of leukocytes. These rapid innate immune responses result from the engagement of multiple cellular damage response pathways (Figure 3a). Alum particles induce cathepsin B and cathepsin S-dependent necrotic cell death [99], and alum induced tissue damage is associated with the release of cellular danger molecules including host cell DNA, ATP and uric acid or monosodium urate (MSU) crystals [100–102]. Uptake of alum-associated DNA by activated APCs appears to promote its adjuvant function, since co-injection of DNase diminished T cell priming [100]. Alum activates the NLRP3 inflammasome through multiple different pathways leading to the release of IL-1 β and pyroptosis [103,104] (Figure 3a). However, the role of NLRP3 for alum-mediated adjuvant activity has been disputed [105,106]. Alum is a well-known T_H2 skewing adjuvant (Figure 3a). Interestingly, it has been suggested that its

Figure 3



Proposed modes of action of alum-adjuvanted vaccines *versus* live attenuated yellow fever vaccine (YF-17D).

(a) Alum elicits a multitude of cellular responses with potential effects on adaptive immunity. Alum activates the NLRP3 inflammasome with subsequent release of IL-1 β and IL-18. Cell death and subsequent inflammation contributes to alum’s adjuvant effects. Release of DNA from necrotic cells activates IRF3, potentially via cGAS/STING signaling. Alum mediated release of monosodium urate crystals (MSU), destabilization of lysosomal compartments and release of Cathepsin B (CTSB), as well as mitochondrial reactive oxygen species (ROS) were shown to activate the NLRP3 inflammasome. Alum adjuvanted vaccines induce T_H2 biased immune responses and elevated antibody production. (b) Live attenuated yellow fever vaccine strain YF-17D is sensed in a MyD88/TIRAP-dependent manner. Activation of TLR7, TLR8, TLR9 (and TLR2), as well as RIG-I-like receptors by YF-17D has been demonstrated, leading to the production of IFN α , IL-12 and various other cytokines. Inactivation of YF-17D by heat or UV radiation strongly diminishes cytokine responses. YF-17D induces polyfunctional T cell responses leading to robust, long-lasting immunity. Pathways whose role is not fully characterized are depicted in grey symbols and grey dotted lines.

T_H2 -bias is only partially dependent on IL-4 and IL-13 [107]. Early activation of eosinophils and mast cells contributes to local inflammation, yet appears dispensable for T_H2 cell priming [105], whereas cathepsin-dependent cell death, and MSU-mediated activation of DCs contributes to T_H2 responses [99,102]. Despite recent advances, the oldest vaccine adjuvant continues to keep its well-hidden secrets.

Setting aside alum's long history as a successful vaccine adjuvant, it seems conceptually counterintuitive to mobilize type-2 immune responses that evolutionarily arose to fight off parasites, for the purpose of protecting against viral and bacterial pathogens. We believe it is time to move on to more targeted approaches that better mimic the causative pathogen, in order to activate more appropriate modes of immunity, particularly T_{FH} and CTL responses.

Live versus dead: *vita*-PAMPs and their function in vaccine responses

LAVs against yellow fever or measles are famously efficacious, providing lifelong immunity upon single-dose immunization, with estimated antibody half-lives of up to 200 years [108,109]. A similar level of protection is rarely achieved with non-live vaccines. In fact, early immunization trials with an inactivated measles virus (MV) vaccine yielded poor efficacy and rapidly waning antibody titers [110]. Inanimate vaccines, such as HBV vaccines, generally require booster immunizations and they are poor inducers of $CD8^+$ cytotoxic T lymphocyte (CTL) responses. Consequently, several new LAVs, for example against Ebola or Dengue virus, are currently in clinical testing with very promising results [111,112]. Despite their unrivalled efficacy and their wide-spread use for generations, surprisingly little is known about the modes of action of the most frequently used LAVs. Their ability to self-propagate and prolong antigen presentation was long thought to be the sole reason for the superiority of live vaccines over inanimate ones, yet the critical role for differential innate immune activation may have been underestimated [113]. Live vaccines, by virtue of their microbial origin, contain natural PAMPs and exert inherent adjuvant activity, whereas subunit vaccines require exogenous adjuvants, which almost always are non-microbial alum and/or oil-in-water emulsions. Despite the apparent importance of innate immune signals, we know relatively little about the sensing receptors for most LAVs; with the exception of the yellow fever vaccine YF-17D, which has been studied in greater detail [114–116]. Live YF-D17 was shown to activate TLR2, -7 and -9 in mice, and TLR8 in human cells [114] (Figure 3b). YF-17D vaccination in humans elicits a transcriptional signature associated with anti-viral innate immunity and IFN signaling, and pathway analyses suggested an involvement of TLR7 and RIG-I like receptors in pDCs [116,117]. Bruni *et al.* demonstrated that YF-17D

differentially engages TLR7 or RIG-I in pDC, depending on the viral route of entry [118]. Cell-free YF-17D particles activate RIG-I, which requires viral replication, whereas cell associated YF-17D is sensed via TLR7 [118]. Importantly, cytokine responses in human and murine DCs are abrogated by UV-inactivation or heat-inactivation of the virus [114,118] (Figure 3b).

Epidemiological and experimental data have long suggested that live microorganisms induce more robust immune responses compared to their inactivated counterparts, even though both contain considerable amounts of PAMPs [110,119]. Several studies have reported a superior ability of viable *versus* killed bacteria to elicit protective immunity [120–122]. The failure of heat killed *Listeria monocytogenes* to prime protective effector T cell-responses in mice has been attributed to differential DC activation by live and killed bacteria [123,124]. Indeed, APCs possess an inherent capacity to discriminate live from inanimate microbial matter [125,126]. Detection of live avirulent bacteria elicits more robust innate immune responses due to the presence of so-called 'viability associated PAMPs' (*vita*-PAMPs), which alert the host immune system to the presence of viable microorganisms [81,125]. Bacterial RNA was identified as the first *vita*-PAMP, which serve as an indicator of elevated infectious threat, thus warranting more vigorous immune responses compared to an encounter with dead microbes or soluble components thereof [26,125]. Human and porcine APC discriminate live from dead bacteria via TLR8-dependent recognition of bacterial RNA; and activation of TLR8 in APC by live bacteria, bacterial RNA, or nucleoside analogs was identified as a potent driver of T_{FH} cell differentiation and vaccine responses [81]. Domestic pigs immunized with a live attenuated *Salmonella* Typhimurium vaccine, but not with a heat inactivated version of the same vaccine, showed increased T_{FH} cell and antibody responses. A hypermorphic TLR8 single-nucleotide polymorphism (SNP) was associated with improved immunity in response to bacillus Calmette-Guérin (BCG) vaccination, a LAV against tuberculosis [81]. Polymorphisms in TLR8 have also been associated with altered immune responses to the measles vaccine [127]. Live bacteria and bacterial RNA also enhanced T_{FH} and antibody responses in mice, albeit through a TRIF-IFN β -IL1 β axis and not via TLR8, which is dysfunctional in mice [78]. These and other critical differences in the PRR repertoire and innate immune cell composition exemplify potential limitations in the transferability of data from mouse adjuvant studies to human vaccines. A recent study compared a new nanoencapsulated malaria vaccine candidate (Pfs25) adjuvanted with either CpG (TLR9), R848 (TLR7/8), or GLA-LSQ (TLR4), to a non-encapsulated GLA-LSQ adjuvanted vaccine in non-human primates [18]. All three nanoparticle vaccines strongly enhanced Pfs25-specific antibody-titers and antibody-avidity and plasma blast

responses in non-human primates. This supports the idea that particulation of PAMPs and antigens may increase their reactogenicity by mimicking direct microbial contact [26]. Numbers of circulating IL-21 producing T_{FH}(-like) cells were comparable in response to all three TLR-adjuvanted vaccines. Notably though, CXCL13 plasma levels, which are good correlates of GC T_{FH} cell numbers and GC activity, were strongly increased in the R848 group [18,128]. Antibody half-life was also significantly prolonged in response to the R848 containing, and to a lesser extent to the CpG-adjuvanted vaccine [18]. Another recent study found that TLR7/8 agonist 3M-052-SE strongly increases the breadth and functionality of HIV *env*-specific antibody responses compared to TLR4 agonist GLA-SE or alum in infant Rhesus Macaques [129]. In summary, these recent results suggest that activation of endosomal TLRs, particularly TLR8, delivers potent stimuli for T_{FH} cell driven long-lived, high-affinity antibody responses. Some LAVs have been shown to activate similar pathways, which may help to explain their outstanding efficacy and long-lived protection.

Outlook: nucleic acid vaccines as LAV mimics?

It was first observed in the early 1990s that injection of DNA molecules encoding a whole gene lead to significant protein expression *in vivo* [130]. This set off efforts to develop a new generation of vaccines based on antigen-coding nucleic acids. Safety and feasibility of the approach was first demonstrated with an HIV *env*-encoding and *rev*-encoding DNA vector, which showed good tolerability and some correlates of immunogenicity [131]. A recent phase-I trial demonstrated the immunogenicity and safety of a rabies mRNA-vaccine candidate, with induction of boostable rabies-specific antibodies and increased numbers of rabies-specific CD4 T cells [132]. Self-replicating dendrimer-RNA nanoparticle vaccines have recently been shown to elicit robust protective immune responses against experimental influenza and Ebola virus infection in pre-clinical mouse models [133]. Conceptually, RNA-based vaccine approaches mimic several fundamental aspects of LAVs, such as prolonged antigen expression and presentation via MHC-I, and stimulation of nucleic acid sensors, without the risks of causing infection or genomic integration [134]. Incorporated features of microbial RNA would likely be recognized as *vita*-PAMPs, which might mobilize immune responses reminiscent of those mounted against LAVs [126]. Successful implementation of RNA-vaccines will require a perfect balance between their immunostimulatory capacity and efficient antigen translation. These new platforms are still in early development, but they may indeed represent a viable path toward combining the outstanding immunogenicity of LAVs with the superior safety profile of non-live vaccines.

Concluding remarks

Magnitude and quality of vaccine responses are critically shaped by innate immune receptors. Engagement of nucleic acid sensing PRRs, particularly endosomal TLRs, efficiently boosts humoral and cellular immune responses to vaccine antigens. As foreign nucleic acids are indicative of microbial infection, their detection may mobilize pathways that are more similar to those activated by LAVs than by common inanimate subunit vaccines. Despite their extraordinary efficacy, mechanistic insights into LAV-induced innate immune responses are relatively scarce. Information of this nature would be critical to better understand their mechanisms of action, which may facilitate the improvement of subunit vaccines. Besides superior efficacy, increased longevity of protection is the most notable difference between LAVs and non-live vaccines. However, most adjuvant studies, including our own, often merely compare magnitudes of responses; and experimental and clinical vaccine studies are often not designed to compare durability of protection, thereby missing a key feature of the most successful vaccines. In order to improve vaccine efficacy and safety, it would be desirable to develop non-live vaccines, which closely resemble the immunological properties of LAVs. Nucleic acid-based vaccines might fit this bill as they offer the prospect of simultaneously delivering self-amplifying antigen and tailored PRR ligands.

Conflict of Interest

Nothing declared.

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