Toll-like receptor-agonist-based therapies for respiratory viral diseases: thinking outside the cell

Jason L.N. Girkin1,2, Steven Maltby2 and Nathan W. Bartlett 1,2

1Viral Immunology and Respiratory Disease Group, University of Newcastle and Hunter Medical Research Institute, Newcastle, Australia. 2Priority Research Centre for Healthy Lungs, University of Newcastle and Hunter Medical Research Institute, Newcastle, Australia.

Corresponding author: Nathan W. Bartlett (Nathan.Bartlett@newcastle.edu.au)

Abstract

Respiratory virus infections initiate in the upper respiratory tract (URT). Innate immunity is critical for initial control of infection at this site, particularly in the absence of mucosal virus-neutralising antibodies. If the innate immune response is inadequate, infection can spread to the lower respiratory tract (LRT) causing community-acquired pneumonia (as exemplified by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019). Vaccines for respiratory viruses (influenza and SARS-CoV-2) leverage systemic adaptive immunity to protect from severe lung disease. However, the URT remains vulnerable to infection, enabling viral transmission and posing an ongoing risk of severe disease in populations that lack effective adaptive immunity.

Innate immunity is triggered by host cell recognition of viral pathogen-associated molecular patterns via molecular sensors such as Toll-like receptors (TLRs). Here we review the role of TLRs in respiratory viral infections and the potential of TLR-targeted treatments to enhance airway antiviral immunity to limit progression to severe LRT disease and reduce person-to-person viral transmission. By considering cellular localisation and antiviral mechanisms of action and treatment route/timing, we propose that cell surface TLR agonist therapies are a viable strategy for preventing respiratory viral diseases by providing immediate, durable pan-viral protection within the URT.

Introduction

Infection-induced severe respiratory diseases (even prior to the coronavirus disease 2019 (COVID-19) pandemic) are amongst the top contributors to death and disability in adults and children globally [1], causing an estimated 4 million deaths annually [2]. The World Health Organization states that community-acquired pneumonia and other infection-induced lower respiratory diseases were the fourth leading cause of death in 2019 worldwide [3]. Viral infection constitutes the most significant cause of infection-induced respiratory disease and can be attributed to a vast number of viral strains/subtypes from nine different families of respiratory viruses; namely, respiratory syncytial virus, influenza, parainfluenza, rhinovirus (RV), adenovirus, coronavirus, metapneumovirus and bocavirus [4]. Influenza infection alone causes an estimated 250000–500000 deaths globally and USD 71–167 billion in associated costs annually [5]. These data pre-date the ongoing and growing impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/COVID-19 pandemic.

Innate immune-mediated protection from virus infections

Innate immunity is not constrained by the need to select antigen-specific lymphocytes, instead employing ubiquitously and constitutively expressed pathogen recognition receptors (PRRs), such as Toll-like receptors (TLRs). TLRs activate innate immunity by binding pathogen-associated molecular patterns.
(PAMPs) [6]. TLRs on the cell surface (TLR1/2, TLR2/6, TLR4, TLR5 and TLR10) are constantly exposed to extracellular stimuli and regulate immune activation by maintaining quiescence during exposure to innocuous environmental molecules and commensal microbes [7]. The capacity for cell surface TLRs to differentiate ‘background environmental and commensal microbial noise’ from PAMPs is critical for homeostasis. Other TLRs (TLR3, TLR7/8 and TLR9) are localised intracellularly in endosomes and predominantly detect RNA- or DNA-based molecular structures associated with pathogens. Activation of intracellular TLRs typically induces a potent immune response mediated by type I interferons (IFNs) and inflammatory cytokines. When delayed and over-exuberant, this inflammatory response can contribute to severe respiratory viral illnesses such as COVID-19 [8] and avian influenza [9].

**PRRs – TLRs**

A key first step in immune activation is detection of pathogens via PRR binding of PAMPs, by both non-immune cells (e.g. epithelial cells) and immune cells (e.g. macrophages, neutrophils). Recognition triggers a “danger signal”, activating a pro-inflammatory cascade that recruits and activates innate and adaptive immune cells [10, 11].

The first-described PRRs were the TLRs, with the discovery in 1998 that mammalian TLR4 binds the bacterial component lipopolysaccharide (LPS) [12]. It is now recognised that the human genome encodes 10 TLRs (TLR1–10), which recognise a range of PAMPs across all groups of pathogens. TLRs can be classified into two groups based on their cellular localisation (cell surface versus intracellular), which reflect the type of pathogen recognised and their role in regulating and triggering innate immunity. The current review focuses on TLRs, with a focus on the role of each TLR in viral infection and their suitability as therapeutic targets. A summary of TLR ligands, cellular distribution and effects of agonist stimulation is provided in table 1. While a range of additional novel treatment approaches are emerging (e.g. miRNAs, nanodrugs, etc.), these have been extensively reviewed elsewhere and are beyond the scope of the current review.

**Innate immunity to respiratory virus infection**

Respiratory viruses typically enter the body via the nose and mouth in droplets ejected from the upper respiratory tract (URT) of an infected person. Following transmission, viral infection of airway epithelial cells (AECs) usually (but not always) triggers parallel signalling pathways to stimulate production of IFNs (type I and type III IFNs) and inflammatory cytokines via activation of IFN regulatory factors (IRFs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [13]. Timely expression of cytokines and IFNs, growth factors and chemokines rapidly mobilises a coordinated mucosal immune response that contains the infection to the URT and initiates adaptive immunity [13]. This immune response also causes symptoms generally referred to as the “common cold”. Virus-induced growth factors and chemokines typically increase neutrophil numbers in blood and sputum [14]. However, virus infection, particularly RV, can also induce eosinophil recruitment in the context of allergic airways diseases such as asthma and lead to an exacerbation [15, 16]. Innate type I and type III IFNs have a range of complex antiviral effects, which are carefully coordinated in response to virus infection [17]. Of relevance to respiratory virus infection, type III IFN-λs are the first IFNs produced upon recognition of virus-associated PAMPs before viral entry [18] and have a key role in local suppression of viral replication by inducing AEC antiviral responses without stimulating systemic inflammation [19]. In contrast, type I IFNs are activated later during virus infection and enhance both antiviral and pro-inflammatory responses that can induce both local (airway) and systemic inflammation [19].

Recognition of PAMPs by TLRs activates IRF- and NF-κB-mediated intracellular signalling pathways [20]. Individual TLRs recruit adaptor molecules containing Toll-IL-1R (TIR) domains and stimulate downstream activation of signalling pathways (figure 1). The exact response induced depends on host factors such as the adaptor molecules recruited, the intensity of TLR activation and the TLR-expressing cell type [21]. The response is also shaped by viral immune evasion genes that interfere with all major components of antiviral innate immune activation pathways identified [22].

Sub-optimal antiviral immune responses characterised by both blunted/reduced [23, 24] and/or delayed [25] cytokine production occur in people with underlying inflammatory respiratory diseases (e.g. asthma and chronic obstructive pulmonary disease (COPD)), which limit virus clearance and contribute to exacerbated respiratory disease symptoms. For example, in vitro RV infection of AEC cultures from asthmatic donors produce lower levels of IFN-β and interleukin (IL)-15 with increased viral load, compared to epithelial cells from healthy donors [23, 24]. Further, induction of antiviral cytokine production was delayed in differentiated epithelial cell cultures from donors with asthma or COPD compared to healthy donors, in a physiologically relevant low multiplicity of infection model of RV
Viruses can also inhibit AEC innate immune activation. AECs infected with human β-coronaviruses OC43 and SARS-CoV-2, despite supporting sustained replication, exhibit deficient expression of type I/III IFNs and inflammatory cytokines which is likely to contribute to delayed onset of symptoms and facilitate transmission.

Agonist treatments for viral infection: targeting cell surface TLRs

TLRs localised on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) generally recognise bacterial components, are in constant contact with the surrounding extracellular microenvironment and are involved in maintaining homeostasis [27]. As such, their activation is tightly regulated, to limit unnecessary inflammation (particularly in the gastrointestinal tract [28]). TLR2, TLR4, TLR5 and TLR10 are discussed below. TLR1 and TLR6 function as co-receptors with TLR2, and as such have been included in the TLR2 section.

**TLR2**

**TLR2 activation by virus infection**

TLR2 was initially identified as a pathogen receptor for bacterial cell-wall components (e.g. peptidoglycan [29]), but is now recognised to be activated by virus components. TLR2 localises to the plasma membrane and forms heterodimers with TLR1 or TLR6, to recognise a broad repertoire of PAMPs (table 1) via a common signalling pathway regardless of the specific TLR2 heterodimer activated [30] and inducing similar gene expression signatures [31].

TLR2 has more recently been identified as involved in innate immune activation to a diverse range of viruses. Type-I IFN responses to the poxvirus vaccinia virus (large enveloped virus with double stranded DNA genome) are dependent on internalisation and activation of TLR2 on inflammatory monocytes [32].

---

**TABLE 1 Toll-like receptors (TLRs), known ligands, cellular localisation and effects of activation**

| Receptor | Cellular localisation | Ligand | Agonist | Evidence from agonist treatment of virus infection |
|----------|-----------------------|--------|---------|--------------------------------------------------|
| TLR2 (as heterodimer with TLR1 or TLR6) | Cell surface | Pam$_2$Cys | Acylated lipopeptides | Type-I IFN responses to vaccinia |
| | | MALP-2 | Palmitic acid | Protects from lethal influenza infection with no effect on adaptive immune responses |
| | | RV VP4 capsid | LP-1 | Intranasal treatment limits influenza spread to lower airways |
| | | SARS-CoV-2 envelope protein | Pam$_2$Cys | Reduces upper respiratory tract viral shedding in SARS-CoV-2 challenge model |
| | | | PEG-diacylated lipopeptide | Induces innate immune priming, enhances early type-III IFN responses and reduces viral load after RV infection |
| | | | Pam2C-SK4 | Reduces upper respiratory tract viral shedding in SARS-CoV-2 challenge model |

**TLR3**

| | Intra | ssRNA | Poly I:C | Protection from influenza yellow fever, Rift Valley fever, rabies |
| | | | PIKA | Adjuvant protecting from HBV |

**TLR4**

| | Cell surface | LPS | RSV F | Protects from lethal influenza infection |
| | | EBOV G | VSV G | |
| | | | DENV NS1 | |
| | Flagellin | Lipopolysaccharide | FimH | Vaccine adjuvant for influenza |
| | | | MPLA | Protects from CMV and influenza infection |

**TLR5**

| | Cell surface | Flagellin | R-848 | Vaccine adjuvant for influenza |
| | | | Imiquimod | Protects from CMV and influenza infection |

**TLR7/TLR8**

| | Intra | ssRNA | R-848 | Vaccine adjuvant for influenza |
| | | | Imiquimod | Antiviral approach for HBV/HCV |

**TLR9**

| | Intra | Unmethylated DNA | CPG10101 | Protects from HCV infection |
| | | | CPG7909 | Vaccine adjuvant for HIV |

**TLR10**

| | Cell surface | Unknown | | |

CMV: cytomegalovirus; DENV NS1: dengue virus non-structural protein 1; EBOV G: Ebola virus glycoprotein; FimH: fimbriae H protein; HBV: hepatitis B virus; HCV: hepatitis C virus; IFN: interferon; Intra: intracellular/endosomal; LPS: lipopolysaccharide; MALP: macrophage-activating lipopeptide; MPLA: monophosphoryl lipid A; PEG: pegylated; Poly I:C: polynosinic-polycytidylic acid; Poly IC:LC: polynosinic-polycytidylic acid stabilised with poly-L-lysine and carboxymethylcellulose; RSV F, respiratory syncytial virus via the fusion protein; RV: rhinovirus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VSV G: vesicular stomatitis virus glycoprotein.

Infection [25]. Viruses can also inhibit AEC innate immune activation. AECs infected with human β-coronaviruses OC43 and SARS-CoV-2, despite supporting sustained replication, exhibit deficient expression of type I/III IFNs and inflammatory cytokines which is likely to contribute to delayed onset of symptoms and facilitate transmission [26].
TLR2 is also implicated in the response to one of the smallest RNA viruses – RV. Myristoylated RV VP4 capsid protein interacts with TLR2 during cell entry and induces pro-inflammatory cytokine gene expression in vitro [33]. The envelope protein of β-coronaviruses, including SARS-CoV-2, is also sensed by TLR2. In this context, it has been suggested that TLR2-mediated inflammation contributes to cytokine storm-induced mortality in COVID-19 patients [34]. This might be true for the lower respiratory tract (LRT) and late-stage severe COVID-19 but the opposite appears to be the case for early URT responses to SARS-CoV-2, where innate immunity is significantly dampened and thought to underpin mild/asymptomatic illness and promote transmission [35]. With no pre-existing immunity, this inadequate innate immune response during the initial days following infection almost certainly enables SARS-CoV-2 transmission and contributed to the current pandemic disease.

**Therapeutic targeting of TLR2**

Administration of the TLR2 agonist Pam$_2$Cys to the lung protects against lethal influenza infection [36], but had no effect on subsequent development of adaptive immune responses [37]. Further, TLR2 agonist treatment via intranasal delivery specifically prevented the spread of influenza infection to the lower airways [38]. We recently reported that prophylactic intranasal administration of a pegylated Pam$_2$Cys analogue reduced RV lung viral load in mice in vivo and in air–liquid interface-cultured primary human bronchial epithelial cell cultures in vitro [39]. Prophylactic treatment was effective when administered 7 days before infection and was associated with innate immune activation, increased IFN expression and inhibition of neutrophilic inflammation in vivo [39]. Similarly, treatment in vitro primed innate immunity defined by upregulated IFN-λ, chemokine and anti-microbial gene expression and an accelerated response to infection, enriched for NF-κB-regulated anti-microbial genes. This boosted response resolved rapidly, coincident with reduced viral load [39]. Of note, protection also occurred in epithelial cell cultures derived from donors with asthma [39], which have delayed responses to RV infection [25]. Further, we recently reported that prophylactic intranasal administration of the related TLR2/6 agonist (INNA-051) reduced levels of SARS-CoV-2 shedding in a ferret infection model [40].
These data highlight that TLR2 promotes innate immune responses to multiple respiratory viruses (influenza, RV and SARS-CoV-2) and that prophylactic TLR2 activation can prime airway immunity for an accelerated response to infection that is primarily associated with early induction of IFN-λ (rather than increased IFN-β expression). A schematic overview of the proposed mechanism of action of TLR2-mediated antiviral responses is included in figure 2.

**TLR4**

**TLR4 activation by virus infection**

Prior to ligand engagement, TLR4 localises to the cell surface and is best known for binding to the bacterial cell-wall component LPS, a ligand predominantly associated with Gram-negative (and some Gram-positive) bacteria [12]. TLR4 is unique in that it can be internalised and signal from endosomes where it can sense viral glycoproteins and activates multiple intracellular signalling pathways [41]. CD14-mediated binding of LPS and subsequent interaction with TLR4 induces TLR4 dimerisation and downstream Toll-IL 1 receptor domain containing adaptor protein (TIRAP)/MyD88 activation [42]. TLR4 also migrates to endosomal membranes (in association with CD14), where ligand binding results in TRIF-related adaptor molecule/TIR-domain-containing adaptor-inducing interferon-beta (TRAM/TRIF), IRF-3 signalling and IFN-β expression [43]. Thus, TLR4 intracellular endosomal signalling downstream of TLR4 activation stimulates the production of type I IFNs via NF-κB and IRF-3-mediated processes [44]. Like TLR2, TLR4 activation was not previously thought to occur during virus infection. However, a growing list of viruses is now recognised to induce inflammatory responses via TLR4 including respiratory syncytial virus (RSV) via the fusion protein, Ebola virus glycoprotein, vesicular stomatitis virus

**Absence of infection**

| Macrophages/ APCs | T-cells | B-cells |
|-------------------|---------|---------|
| TLR2 agonist      | IFN-λ, chemokines antimicrobials | TLR2 agonist TLR2/TLR6 |

**Upon viral infection**

| IFN-λ, IFN-γ, chemokines | Antimicrobials | Memory T-cells | IgA+IgG |
|---------------------------|----------------|----------------|---------|
| TOLLIP                     | NF-κB          | APCs           | HLA     |
| SOCS1                      |                |                | MHC     |
| IDO-1                      |                |                |         |
| Chemokines                 |                |                |         |
| JAK/STAT                   | Viral RNA      | ISGs           |         |
| ISGs                       | IFN-λ          |                |         |
| IL-28R1/IL-10R2            |                |                |         |
| IFN-γ                      |                |                |         |
| IFN-λ                      |                |                |         |
| NF-κB                      |                |                |         |
| IRF7                       |                |                |         |

**Adaptive immunity (speculative)**

| Memory T-cells | IgA+IgG | APCs | HLA | MHC |
|----------------|---------|------|-----|-----|
|                |         |      |     |     |

**FIGURE 2** Proposed Toll-like receptor 2 (TLR2) agonist treatment mechanism of action. TLR agonists engage the TLR2 heterodimer receptor inducing expression of interferon (IFN)-λ, antimicrobial proteins (e.g. indoleamine 2,3-dioxygenase 1 (IDO1)) and chemokine expression through nuclear factor-xB (NF-xB) signalling. Chemokines release recruited lymphocytes, macrophages and antigen-presenting cells (APCs) to the respiratory mucosa to establish innate immune priming. Virus infection after TLR2 agonist treatment increases NF-xB and (IFN regulatory factor 7 (IRF7) signalling, resulting in early expression of IFN-λ following recognition of viral RNA by endosomal TLR3 (double-stranded intermediate) and/or TLR7/8 (single-stranded RNA). Synergy between IFN-λ-mediated IFN-stimulated genes (ISGs), antimicrobial factors, and mucosal lymphocyte activation reduce viral load. Transcriptome data indicate enhanced antigen presentation leading to humoral and cell mediated adaptive immunity. JAK/STAT: Janus-associated kinase/signal transducer and activator of transcription; HLA: human leukocyte antigen; Ig: immunoglobulin; IL: interleukin; MHC: major histocompatibility complex; SOCS1: suppressor of cytokine signalling; TOLLIP: Toll-interacting protein. Image generated using BioRender.
glycoprotein and the dengue virus non-structural protein 1 [45]. Excessive and/or chronic TLR4 activation can contribute to severe inflammatory disease, including acute respiratory distress syndrome (ARDS) and systemic organ failure.

**Therapeutic targeting of TLR4**

Prophylactic stimulation of TLR4 via fimbiae H protein administration protected against lethal influenza A virus infection in a mouse model [46]. Protection was associated with local innate immune activation, increased neutrophil infiltration to the airway lumen and increased levels of inflammatory cytokines tumour necrosis factor-α (TNF-α), RANTES and IL-12 in bronchoalveolar lavage fluid [46]. Administration of monophosphoryl lipid A (MPLA; another TLR4 agonist) also enhanced both mucosal and systemic immune responses to vaccine components, and MPLA treatment prior to infection protected against lethal influenza infection [47, 48]. Whilst there is some encouraging data to support development of TLR4 agonists as a protective treatment for respiratory virus infection, more research has focussed on TLR4 blockade treatment to suppress viral inflammation-induced acute lung injury and ARDS [49]. TLR4’s ability to signal from both the plasma membrane and endosomes and reliably controlling immune activation (production of inflammatory cytokines and IFN-β) represent challenges for TLR4-agonist based therapies as an approach to prevent and/or treat respiratory virus infection. TLR4-targeted treatment will require careful consideration of therapeutic windows/timing to provide protection from disease while limiting unintended severe inflammatory disease.

**TLR5**

**TLR5 activation by infection**

TLR5 localises to the cell surface and recognises flagellin, a component of bacterial flagella, though there is also clear evidence that TLR5 is involved in the immune response to cytomegalovirus (CMV) [50].

**Therapeutic targeting of TLR5**

Flagellin is highly immunogenic and has been used as a vaccine adjuvant to stimulate antiviral immunity, via fusion to influenza A and influenza B antigens [51–53]. Flagellin administration reduced influenza A virus replication, independently of type I IFN responses and IL-22 signalling, and increased the efficacy of Oseltamivir treatment [54]. TLR5 ligands have also been assessed as standalone prophylactic treatments for CMV infection in mouse models, where administration reduced viral load in the liver, increased cytotoxic natural killer (NK) cell activity and increased numbers of IFN-γ, granzyme B- and CD107a-producing NK cells [55]. TLR5 ligand administration is a potent adjuvant when administered to the airways [56]. However, TLR5 is quickly degraded in the lung and signalling through TLR5 in the lung is regionalised [56]. Thus, TLR5 agonists may promote antiviral responses in certain settings, but may have limited utility for the treatment of respiratory virus infections.

**TLR10**

**TLR10 activation by virus infection**

TLR10 is an orphan receptor lacking a known distinct agonist and is only present as a pseudogene in mice, which has made it difficult to characterise the function of TLR10. TLR10 gene variant overexpression and/or treatment with anti-TLR10 antibodies had no effect on NF-κB activity in the absence of immunological stimulation [57]. However, TLR10 may be anti-inflammatory upon dimerisation with TLR2 [58]. TLR10 antibody treatment in conjunction with Pam3Cys stimulation of TLR1/2 enhanced production of inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α by peripheral blood mononuclear cells (PBMCs) [58]. Further, silencing TLR10 in monocyte-derived macrophages stimulated greater production of IL-6 [58]. Expression of human TLR10 in mice reduced inflammatory responses (IL-6 and C-X-C motif ligand 1 (CXCL1)/ keratinocytes-derived chemokine (KC)/murine IL-8) to systemic Pam3Cys administration. In B-cells, antibody-mediated engagement of TLR10 inhibited B-cell proliferation, cytokine production and signal transduction, and TLR10 transgenic mice demonstrated diminished antibody responses to T-cell-dependent and -independent antigens [59]. Dendritic cell (DC) maturation markers and capacity to activate T-cells were reduced upon TLR10 antibody treatment of human monocytes cultures and B-cells after TLR4 or TLR8 stimulation [60]. In addition, stable TLR10 knockdown in a human monocyte cell line reduced cytokine production in response to agonist stimulation of TLR2/6 heterodimers and TLR5 [61].

In the context of virus infection, TLR10 expression was linked to the production of cytokine and IFN responses in vitro [62]. TLR10 expression was induced by influenza infection (H1N1 and H5N1) in primary human macrophages and THP-1 cells (human monocyte cell line) [62]. Virus replication (de novo protein synthesis) and soluble factors induced by virus infection both induced TLR10 expression, and experimental reduction of TLR10 expression resulted in suppressed IFN and cytokine responses to influenza A virus [62].
Therapeutic targeting of TLR10
Due to the lack of a specific ligand for TLR10, the absence of clear-cut signalling pathway and conflicting reported roles for TLR10 function, TLR10 is not currently a likely target for treatment approaches.

Agonist treatments for viral infection: targeting intracellular-endosomal TLRs
TLRs located on endosomal membranes within intracellular organelles, including TLR3, TLR7, TLR8 and TLR9, directly recognise motifs within viral nucleic acids [63]. Recognition of viral-associated ligands (or intracellular exposure to endogenous ligands as a result of infection) leads to rapid and potent activation, which initiates innate antiviral immunity through IFN production [64]. Viruses with an ssRNA genome that enter cells via receptor-mediated endocytosis and replicate in the cytoplasm (e.g. RV, paramyxoviruses such as RSV, coronaviruses) generate both single-stranded and double-stranded RNA molecules during their replication cycle. SsRNA directly activates intracellular TLR7/8, while dsRNA is recognised by TLR3. Influenza is somewhat different, being the only RNA-based respiratory virus that replicates in the nucleus. Influenza ssRNA and dsRNA intermediates are sensed by intracellular TLR7 and TLR3, respectively (as well as by additional cytoplasmic PRRs, such as RIG-I and MDA-5) [65, 66].

Activation of intracellular TLRs requires appropriate localisation of the agonist. Drug delivery therefore must be considered. Further, stimulation of intracellular TLRs is more likely to trigger intense pro-inflammatory responses [67–69], which may contribute to unintended pathology – particularly in clinical scenarios where excessive inflammation is the primary driver of disease such as advanced, severe LRT viral illness and viral exacerbation of asthma or COPD.

TLR3

TLR3 activation by virus infection
TLR3 localises to the endosomal membrane and recognises dsRNA, a feature of many virus replication cycles [70]. TLR3 activation stimulates NF-kB activation and downstream antiviral type I IFN production [70]. TLR3 signalling is also activated by exposure to the synthetic molecule Poly I:C (polynosinic:polycytidylic acid), a chemically stabilised dsRNA analogue.

Therapeutic targeting of TLR3
Numerous studies have demonstrated effects of TLR3 stimulation on virus infection outcomes. Treatment with PIKA, a stabilised derivative of Poly I:C, protected mice from influenza A virus infection (including the 2009 pandemic H1N1 virus) [71]. Protection was associated with increased TNF-α, IFN-γ, CXCL1 (mouse IL-8/KC), IFN-β and recruitment of interstitial macrophages, neutrophils and plasmacytoid dendritic cells (pDCs) [71]. Administration of PIKA or Poly I:C as adjuvants protected against hepatitis B virus (HBV), associated with enhanced cellular and humoral immune responses [72, 73]. TLR3 stimulation via polynosinic-polycytidylic acid stabilised with poly-L-lysine and carboxymethylcellulose (Poly IC:LC) administration protected mice and rhesus monkeys from yellow fever, Rift Valley fever and rabies virus infections and also provided protection against multiple, lethal influenza strains [65, 74]. These studies demonstrate potential for the application of TLR3 agonists for protection against viral infection, however they also identified potential safety concerns.

Toxic effects of Poly IC:LC administration have been observed in animal models (e.g. hypothermia and weight loss) which could limit their clinical utility. These effects were mitigated by formulating Poly IC:LC within liposomes, which also enhanced protection from lethal influenza (PR8) infection in mice [75, 76]. Thus, TLR3 agonists have a strong potential to enhance antiviral responses, although careful consideration will be required to determine dosage and treatment regimens to provide protection, while limiting toxic side-effects and excessive inflammation. These considerations will be particularly important when considering TLR3 agonist treatment for patients with pre-existing inflammatory airways diseases.

TLR7/8

TLR7/8 activation by virus infection
TLR7 and TLR8 localise to the endosomal membrane and detect guanosine and uridine-rich ssRNA in the cytosol of infected cells during infection by a range of viruses including human immunodeficiency virus (HIV), influenza virus and RV [77, 78]. TLR7 is activated in immune phagocytes, when viral nucleic acids colocalise with TLR7 following endo-lysosomal fusion [79]. Upon activation, TLR7 signals through MyD88-dependent pathways to induce IFN production [80] via the transcription factor, IRF-7 [81–83], a master regulator of type I IFNs [84]. Although TLR7 is expressed in multiple cell types, pDCs are the predominant source of TLR7-induced IFN [85].

https://doi.org/10.1183/16000617.0274-2021
Of relevance to respiratory virus-induced exacerbations of lung disease, several studies have reported impaired TLR7 signalling associated with deficient IFN production in people with asthma during RV infection. RV infection of bronchoalveolar lavage or bronchial epithelial cells isolated from people with asthma results in deficient IFN production, compared to infection of cells from healthy controls [23, 86]. Further, IFN production in response to TLR7 stimulation is reduced in PBMCs isolated from people with asthma, compared to cells isolated from healthy controls [87]. Interestingly, people with well-controlled asthma are less likely to exhibit IFN-deficiency [88]. Impaired TLR7 function may also contribute to more severe RV infections in the context of asthma, based on data obtained from mouse models. Eosinophilic inflammation induced by IL-5, suppressed TLR7 expression in a mouse model of allergic airways disease, resulting in less IFN production in response to RV-A1 infection [89]. TLR7 knockout mice (TLR7\(^{-/-}\)) also had decreased IFN responses, exaggerated eosinophilic inflammation and increased airway hyperresponsiveness to methacholine, which was restored by adoptive transfer of TLR7-competent wild-type pDCs [89]. In line with these observations, TLR-agonists can be used to potentiate allergic inflammation or even induce tolerogenic profiles in response to allergens and although this falls outside the scope of this review, this concept has been reviewed elsewhere [90].

**Therapeutic targeting of TLR7/8**

TLR7 and TLR8 activation and downstream IFN production can be induced by exposure to a range of synthetic agonists, including R-848, imiquimod or loxoribine (a synthetic nucleoside) [91, 92]. Because of the pivotal role of TLR7 in immune responses against viruses, TLR7 agonists are currently used as vaccine adjuvants for multiple strains of influenza [93] and being assessed as an antiviral treatment approach for HBV and hepatitis C virus (HCV) infection [94–99]. For example, TLR7 agonist treatment suppressed HBV replication in a HepG2.2.15 cell line [94], provided long-term suppression in HBV-infected chimpanzees [98] and had efficacy in a mouse HCV infection model [99]. TLR7 agonist treatment was relatively well-tolerated in patients with ongoing HBV [97] or HCV infection [96], although treatment did not significantly alter virus levels. In other studies, TLR7 agonists such as PF-4878691 have exhibited a narrow therapeutic range, with severe adverse effects observed with the dose levels required to generate antiviral efficacy mediated by IFN-α induction, leading to early termination of clinical trials [100].

Thus, TLR7 activation is relevant in the context of respiratory disease (i.e., asthma) and respiratory virus infection. TLR7 agonist administration has been assessed for vaccine development and potential antiviral therapy, but not as a therapeutic approach for the prevention of respiratory virus infection in the context of underlying inflammatory disease. TLR7 agonist treatment is also not well suited for treatment of acute, severe respiratory viral lung disease with potential contribution to acute virus-induced immunopathology exemplified by a study that reported blockade of TLR7 reduced mortality in a mouse model of severe influenza. Rather than reducing the innate immune response in AECs, the TLR7 antagonist reduced excessive type I IFN and inflammatory cytokine/chemokine production by pDC and monocytes and this reduced mortality [101]. This also highlights the safety concerns related to bystander immune cell activation by topical (e.g. inhaled) exposure to TLR7 agonists which may be addressed using drug delivery platforms such as nanoparticles that shield the agonist from immune cells and facilitate delivery to virus-infected AECs [102].

**TLR9**

**TLR9 activation by virus infection**

TLR9 localises to the endosomal membrane and recognises microbial DNA containing unmethylated CpG dideoxynucleotides [103, 104]. TLR9 is expressed by a diverse range of immune cells [105, 106] and is activated in response to infection by DNA viruses, including poxviruses [107], herpesviruses [108] and adenoviruses [109]. Overlap between TLR9-expressing cell types and downstream pathology have prompted speculation that TLR9 may mediate the severe symptoms of SARS-CoV-2/COVID-19 infection [110].

**Therapeutic targeting of TLR9**

Synthetic CpG oligodeoxynucleotides have primarily been assessed as vaccine adjuvants [111, 112] and standalone therapies for the treatment of chronic infections (e.g. HCV). Numerous clinical trials assessing TLR9 agonist administration have provided valuable insight into the feasibility and safety of TLR agonists as a treatment approach. Subcutaneous administration of CPG7909 (a synthetic TLR9 agonist) to healthy volunteers promoted systemic Th1 responses, characterised by increased IL-6, IL-12p40, IFN-α, and IFN-inducible chemokines (including CXCL10) [113]. Administration of the TLR9 agonist CPG10101 prior to HCV infection stimulated cytokine production (CXCL10), antiviral responses (IFN-α, oligoadenylate synthetase) and decreased HCV RNA [114]. Treatment was well-tolerated, and the mild adverse events observed were similar to those observed following recombinant IFN-α treatment [115]. Vaccines containing CPG7909 reduced HIV pro-viral load and increase numbers of HIV-specific CD8\(^{+}\) T cells [116].
T-cells [116]. While some clinical trials assessing TLR9 agonists have reported adverse events [117], the majority of the events have been mild (e.g. injection site reactions) and TLR9 agonist treatment has generally been well tolerated. Severe adverse effects (e.g. high-grade neutropaenia and electrolyte disturbances) were reported when TLR9 agonists were paired with chemotherapy for the treatment of advanced non-small-cell lung cancer [118, 119], although these effects have not been observed in subsequent trials [120]. Whether TLR9 agonist administration or the combination therapies applied cause these effects remain unclear.

**Treatment delivery route**

It is important to also consider the route / method of treatment delivery and downstream effects. As outlined above, respiratory virus infections begin upon exposure to virus in URT, resulting in “common cold” symptoms [121]. Severe symptoms leading to community acquired pneumonia are typically associated with subsequent spread of the virus into the lower respiratory tract. Emerging evidence suggests that severe disease may also be triggered by direct droplet aspiration of SARS-CoV-2 [122], although the relative contribution of this route of entry remains uncertain. Treatments which are delivered systemically (e.g. oral delivery and injected vaccines) often fail to establish effective adaptive immune responses in the URT, which has also been reported in primate studies of SARS-CoV-2 vaccines [123, 124]. Targeting the virus at the URT involves directly administering to the nasal mucosa using a nasal spray. Depending on the drug, this has the potential added benefit of limiting systemic exposure and associated adverse events. In particular, chronic fatigue associated with systemic TLR-driven inflammation can be avoided with localised administration, restricted to the URT mucosa [125, 126]. We propose that immune-modulatory treatments which can be delivered and/or targeted directly to the respiratory tract will safely limit virus replication in the early stages of infection, which may reduce peak viral load and reduce virus spread, even where effective vaccines are available. Children are less susceptible to SARS-CoV-2-induced severe illness, likely due (in part) to enhanced innate immune control of viral infection in the URT, with differences in cell populations and IFN-mediated responses observed, in addition to heightened expression of PRRs [127, 128] leading to reduced markers of systemic IFN responses, indicative of better control of the virus in respiratory tract [129]. URT delivery (e.g. nasal spray) has the potential to establish early local immunity, limit infection initiation and reduce progression to LRT disease and person-to-person transmission.

**Prevention of respiratory viral disease**

The phenomenal success of vaccines at preventing human viral diseases (recently exemplified by COVID-19 vaccines) cannot be overstated and is irrefutable evidence that immunity can be harnessed to prevent infectious diseases. Of the 10+ vaccine-preventable viral diseases, only two target respiratory viruses (influenza and COVID-19), though a vaccine to respiratory syncytial virus is on the horizon [130]. This will likely increase in coming years, expedited by the emergence of new vaccine platforms (e.g. mRNA vaccines) and enhanced recognition of the burden of respiratory virus infections. However, several factors limit the availability and efficacy of vaccine approaches for respiratory viral infection.

RV serves as a useful case study for the challenges confronting preventative treatment/vaccine development. RV is the most common human respiratory viral pathogen and causes up to 60% of annual respiratory illnesses globally [131, 132]. Failure to adequately control RV infection to the URT can lead to LRT infection and severe disease, including bronchiolitis and community acquired pneumonia [121]. There are no preventive treatments or vaccines for RV infection and treatment is limited to supportive, non-specific options (e.g. non-steroidal anti-inflammatory drugs [133]).

**Vaccine limitations**

Effective vaccines for respiratory viruses are difficult to develop, particularly for viruses with numerous subtypes and/or genetic/antigenic diversity. Decades of research since the discovery of RV [134] have not yielded an effective vaccine. A major challenge is the large number (>160) of genetically distinct subtypes [135]. While early RV vaccines effectively induced humoral immune responses against specific RV strains [136], they failed to induce broad cross-reactive immunity [137, 138]. While multivalent vaccines effectively generated antibody responses against 25 and 50 RV strains in mice or rhesus macaques, respectively [139], it remains unclear how many subtypes would have to be included to yield broad clinical benefit. Influenza virus can also reshuffle its segmented genome, requiring updated influenza vaccines that protect against new virus strains [140]. Further, influenza A viruses (like β-coronaviruses) reside in animal reservoirs, provide ongoing potential for spill over and emergence of novel human viruses [141]. As vaccine development requires a detailed knowledge of virus genome sequence, structure and replication cycle, vaccine availability lags for new human viruses. Even where vaccines are available,
non-vaccine preventative treatments are important for population groups that are not well protected by vaccination. A broadly effective preventative treatment that leverages innate immunity could address this unmet need.

Conclusions
TLRs are promising therapeutic targets for the prevention of respiratory virus infection. Standalone TLR agonist treatments have demonstrated antiviral effects in a range of infections. We note that careful consideration should be given to the effects of TLR agonist treatment on inflammatory mediators and type I IFN production. Cell surface TLRs that are not classically associated with antiviral virus immunity, particularly TLR2/6, have recently shown promise in pre-clinical studies against multiple different respiratory viruses via innate immune priming of the respiratory mucosa. COVID-19 has reinforced the understanding that innate immunity in the URT plays a key role in disease caused by respiratory viruses and is therefore a viable preventative treatment option, particularly for those at high risk of infection and severe disease.

Provenance: Submitted article, peer reviewed.

Author contributions: All authors contributed to conceptualisation, visualisation, writing – original draft, and writing – review and editing.

Conflict of interest: J.L.N. Girkin reports receiving consulting fees from Ena Respiratory, outside the submitted work. Support for attending meetings and/or travel from Ena Respiratory. Patents issued: US Patent App. 16/495,829, 2020. S. Maltby has nothing to disclose. N.W. Bartlett reports receiving consulting fees from Ena Respiratory, outside the submitted work. Patents issued: Ena Respiratory - Patents PCT/AU2018/050295 Treatment of Respiratory Infection. Stock options held for Ena Respiratory. Receipt of equipment, materials, drugs, medical writing, gifts or other services received from Ena Respiratory: Receipt of TLR2/6 agonist INNA-X.

References
1. Forum of International Respiratory Societies. The Global Impact of Respiratory Disease. 2nd Edn. Sheffield, European Respiratory Society, 2017.
2. Wardlaw TM, Johansson E, Hodge M, et al. World Health Organization & United Nations Children’s Fund (UNICEF). Pneumonia: The Forgotten Killer of Children. Geneva, World Health Organization, 2006.
3. World Health Organization. WHO reveals leading causes of death and disability worldwide: 2000–2019. www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)
4. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11: 373–384.
5. Leisman DE, Ronner L, Pinotti R, et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. Lancet Respir Med 2020; 8: 1233–1244.
6. Schenten D, Medzhitov R. The control of adaptive immune responses by the innate immune system. Adv Immunol 2011: 109: 87–124.
7. Poltorak A, He X, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998; 282: 2085–2088.
8. Kennedy JL, Turner RB, Braciale T, et al. Pathogenesis of rhinovirus infection. Curr Opin Virol 2012; 2: 287–293.
9. Jarjour NN, Germ JE, Kelly EA, et al. The effect of an experimental rhinovirus 16 infection on bronchial lavage neutrophils. J Allergy Clin Immunol 2000; 105: 1169–1177.
10. Calhoun WJ, Dick EC, Schwartz LB, et al. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. J Clin Invest 1994; 94: 2200–2208.
16 Message SD, Laz-Stanca V, Mallia P, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci USA* 2008; 105: 13562–13567.

17 Mezev EV, LeDesma RA, Ploss A. Decoding type I and III interferon signalling during viral infection. *Nat Microbiol* 2019; 4: 914–924.

18 Odendall C, Voak AA, Kagan JC. Type III IFNs are commonly induced by bacteria-sensing TLRs and reinforce epithelial barriers during infection. *J Immunol* 2017; 199: 3270–3279.

19 Galani IE, Triantafyllia V, Eleminiadou E-E, et al. Interferon-λ mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. *Immunity* 2017; 46: 875–890.e6.

20 Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* 2014; 5: 461.

21 Yamamoto M, Takeda K, Akira S. TIR domain-containing adapters define the specificity of TLR signaling. *Mol Immunol* 2004; 40: 861–868.

22 Kikkert M. Innate immune evasion by human respiratory RNA viruses. *J Innate Immun* 2020; 12: 4–20.

23 Wark PAB, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; 201: 937–947.

24 Laza-Stanca V, Message SD, Edwards MR, et al. The role of IL-15 deficiency in the pathogenesis of virus-induced asthma exacerbations. *PLoS Pathog* 2011; 7: e1002114.

25 Veerati PC, Troy NM, Reid AT, et al. Airway epithelial cell immunity is delayed during rhinovirus infection in asthma and COPD. *Front Immunol* 2020; 11: 974.

26 Loo S-L, Wark PAB, Esneau C, et al. Human coronaviruses 229E and OC43 replicate and induce distinct antiviral responses in differentiated primary human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2020; 319: L926–L931.

27 Rakoff-Nahoum S, Paglino J, Es Lam-Varannezh F, et al. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004; 118: 229–241.

28 Biswas A, Wilmsinski J, Forsman H, et al. Negative regulation of Toll-like receptor signaling plays an essential role in homeostasis of the intestine. *Eur J Immunol* 2011; 41: 182–194.

29 Schwandner R, Dziarski R, Wesche H, et al. Peptidoglycan-and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 1999; 274: 17406–17409.

30 Ozinsky A, Underhill DM, Fontenot JD, et al. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 2000; 97: 13766–13771.

31 Farhat K, Riekenberg S, Heine H, et al. Heterodimerization of TLR2 with TLR1 or TLR6 expands the ligand spectrum but does not lead to differential signaling. *J Leukoc Biol* 2008; 83: 692–701.

32 Barbalat R, Lau L, Locksley RM, et al. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol* 2009; 10: 1200–1207.

33 Bentley JK, Han M, Jaipalli S, et al. Myristoylated rhinovirus VP4 protein activates TLR2-dependent proinflammatory cell activation. *Am J Physiol Lung Cell Mol Physiol* 2019; 317: L57–L70.

34 Zheng M, Karki R, Williams EP, et al. TLR2 senses the SARS-CoV-2 envelope protein to produce inflammatory cytokines. *Nat Immunol* 2021; 22: 829–838.

35 Vanderheiden A, Ralfs P, Chirikova T, et al. Type I and type III interferons restrict SARS-CoV-2 infection of human airway epithelial cultures. *J Viral* 2020; 94: e00985-20.

36 Tan AC, Mifsud EJ, Zeng W, et al. Intranasal administration of the TLR2 agonist Pam3Cys provides rapid protection against influenza in mice. *Mol Pharm* 2012; 9: 2710–2718.

37 Mifsud EJ, Tan ACL, Brown LE, et al. Generation of adaptive immune responses following influenza virus challenge is not compromised by pre-treatment with the TLR-2 agonist Pam3Cys. *Front Immunol* 2015; 6: 290.

38 Deliyannis G, Wang CY, McQuilten HA, et al. TLR2-mediated activation of innate responses in the upper airways confers antiviral protection of the lungs. *JCI Insight* 2021; 6: e140267.

39 Girkin J, Loo S-L, Esneau C, et al. TLR2-mediated innate immune priming boosts lung anti-viral immunity. *Eur Respir J* 2021; 58: 2001584.

40 Proud PC, Tsitoura D, Watson RJ, et al. Prophylactic intranasal administration of a TLR2/6 agonist reduces upper respiratory tract viral shedding in a SARS-CoV-2 challenge ferret model. *EBioMed* 2021; 63: 103153.

41 Brubaker SW, Bonham KS, Zanoni I, et al. Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol* 2015; 33: 257–290.

42 Lu Y-C, Yeh W-C, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine* 2008; 42: 145–151.

43 Rajaiah R, Perkins DJ, Ireland DDC, et al. CD14 dependence of TLR4 endocytosis and TRIF signaling displays ligand specificity and is dissociable in endotoxin tolerance. *Proc Natl Acad Sci USA* 2015; 112: 8391–8396.

44 Kagan JC, Su T, Horng T, et al. TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat Immunol* 2008; 9: 361–368.

45 Olejnık J, Hume AJ, Mühlberger E. Toll-like receptor 4 in acute viral infection: too much of a good thing. *PLoS Pathog* 2018; 14: e1007390.
Abdul-Careem MF, Firoz Mian M, Gillgrass AE, et al. FimH, a TLR4 ligand, induces innate antiviral responses in the lung leading to protection against lethal influenza infection in mice. *Antiviral Res* 2011; 92: 346–355.

Baldridge JR, Yorgensen Y, Ward JR, et al. Monophosphoryl lipid A enhances mucosal and systemic immunity to vaccine antigens following intranasal administration. *Vaccine* 2000; 18: 2416–2425.

Persing DH, Coler RN, Lacy MJ, et al. Taking toll: lipid A mimetics as adjuvants and immunomodulators. *Trends Microbiol* 2002; 10: Suppl. 10, S32–S37.

Shirey KA, Blanco JCG, Vogel SN. Targeting TLR4 signaling to blunt viral-mediated acute lung injury. *Front Immunol* 2021; 12: 705080.

Zheng W, Xu Q, Zhang Y, et al. Toll-like receptor-mediated innate immunity against herpesviridae infection: a current perspective on viral infection signaling pathways. *Virol J* 2020; 17: 192.

Taylor DN, Treanor JJ, Sheldon EA, et al. Development of VAX128, a recombinant hemagglutinin (HA) influenza-flagellin fusion vaccine with improved safety and immune response. *Vaccine* 2012; 30: 5761–5769.

Song L, Liu G, Umlauf S, et al. A rationally designed form of the TLR5 agonist, flagellin, supports superior immunogenicity of influenza B globular head vaccines. *Vaccine* 2014; 32: 4317–4323.

Song L, Zhang Y, Yun NE, et al. Superior efficacy of a recombinant flagellin: H5N1 HA globular head vaccine is determined by the placement of the globular head within flagellin. *Vaccine* 2009; 27: 5875–5884.

Georgel A-F, Cayet D, Pizzorno A, et al. Toll-like receptor 5 agonist flagellin reduces influenza A virus replication independently of type I interferon and interleukin 22 and improves antiviral efficacy of oseltamivir. *Antiviral Res* 2019; 168: 28–35.

Hossain MS, Ramachandiran S, Gewirtz AT, et al. Recombinant TLR5 agonist CBLB502 promotes NK cell-mediated anti-CMV immunity in mice. *PLoS ONE* 2014; 9: e96165.

Van Maelle L, Fougeron D, Janot L, et al. Airway structural cells regulate TLR5-mediated mucosal adjuvant activity. *Mucosal Immunol* 2013; 7: 489.

Barreiro LB, Ben-Ali M, Quach H, et al. Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. *PLoS Genet* 2009; 5: e1000562.

Oosting M, Cheng SC, Bolscher JM, et al. Human TLR10 is an anti-inflammatory pattern-recognition receptor. *Proc Natl Acad Sci USA* 2014; 111: E4478–E4484.

Hess NJ, Jiang S, Li X, et al. TLR10 is a B cell intrinsic suppressor of adaptive immune responses. *J Immunol* 2017; 198: 699–707.

Hess NJ, Felicelli C, Grage J, et al. TLR10 suppresses the activation and differentiation of monocytes with effects on DC-mediated adaptive immune responses. *J Leukoc Biol* 2017; 101: 1245–1252.

Le HV, Kim JY. Stable Toll-like receptor 10 knockdown in THP-1 cells reduces TLR-ligand-induced proinflammatory cytokine expression. *Int J Mol Sci* 2016; 17: 859.

Lee SM, Kok KH, Jaume M, et al. Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. *Proc Natl Acad Sci USA* 2014; 111: 3793–3798.

Beutler B. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 2004; 430: 257–263.

Kawai T, Akira S. Innate immune recognition of viral infection. *Nat Immunol* 2006; 7: 131–137.

Wong JP, Christopher ME, Viswanathan S, et al. Activation of toll-like receptor signaling pathway for protection against influenza virus infection. *Vaccine* 2009; 27: 3481–3483.

Le Goffic R, Pothlichet J, Vitour D, et al. Cutting edge: influenza A virus activates TR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *J Immunol* 2007; 178: 3368–3372.

Harris P, Sridhar S, Peng R, et al. Double-stranded RNA induces molecular and inflammatory signatures that are directly relevant to COPD. *Mucosal Immunol* 2013; 6: 474–484.

Wu JJ, Huang DB, Tyring SK. Resiquimod: a new immune response modifier with potential as a vaccine adjuvant for Th1 immune responses. *Antiviral Res* 2004; 64: 79–83.

Horscroft NJ, Pryde DC, Bright H. Antiviral applications of Toll-like receptor agonists. *J Antimicrob Chemother* 2012; 67: 789–801.

Alexopoulou L, Holt AC, Medzhitov R, et al. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 2001; 413: 732–738.

Lau YF, Tang LH, Ooi EE, et al. Activation of the innate immune system provides broad-spectrum protection against influenza A viruses with pandemic potential in mice. *Virology* 2010; 406: 80–87.

Shen E, Li L, Li L, et al. PIKA as an adjuvant enhances specific humoral and cellular immune responses following the vaccination of mice with HBsAg plus PIKA. *Cell Mol Immunol* 2007; 4: 113–120.

Wu J, Huang S, Zhao X, et al. Poly(I:C) treatment leads to interferon-dependent clearance of hepatitis B virus in a hydrodynamic injection mouse model. *J Virol* 2014; 88: 10421–10430.

Wong JP, Saravolac EG, Sabuda D, et al. Prophylactic and therapeutic efficacies of poly(I:C,LC) against respiratory influenza A virus infection in mice. *Antimicrob Agents Chemother* 1995; 39: 2574–2576.

Wong JP, Yang H, Nagata L, et al. Liposome-mediated immunotherapy against respiratory influenza virus infection using double-stranded RNA poly I:CLC. *Vaccine* 1999; 17: 1788–1795.
Wong JP, Christopher ME, Salazar AM, et al. Nucleic acid-based antiviral drugs against seasonal and avian influenza viruses. Vaccine 2007; 25: 3175–3178.

Diebold SS, Kaisho T, Hemmi H, et al. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 2004; 303: 1529–1531.

Heil F, Hemmi H, Hochrein H, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 2004; 303: 1526–1529.

Nishiya T, Kajita E, Miwa S, et al. TLR3 and TLR7 are targeted to the same intracellular compartments by distinct regulatory elements. J Biol Chem 2005; 280: 37107–37117.

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124: 783–801.

Kawai T, Sato S, Ishii KJ, et al. Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 2004; 5: 1061–1068.

Honda K, Ohba Y, Yanai H, et al. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature 2005; 434: 1035–1040.

Honda K, Yanai H, Negishi H, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 2005; 434: 772–777.

Honda K, Taniguchi T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol 2006; 6: 644–658.

Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol 2008; 8: 594–606.

Sykes A, Edwards MR, Macintyre J, et al. Rhinovirus 16-induced IFN-alpha and IFN-beta are deficient in bronchoalveolar lavage cells in asthmatic patients. J Allergy Clin Immunol 2012; 129: 1506–1514.e6.

Forbes RL, Gibson PG, Murphy VE, et al. Impaired type I and III interferon response to rhinovirus infection during pregnancy and asthma. Thorax 2012; 67: 209–214.

Sykes A, Macintyre J, Edwards MR, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. Thorax 2014; 69: 240–246.

Hatchwell L, Collison A, Girkin J, et al. Toll-like receptor 7 governs interferon and inflammatory responses to rhinovirus and is suppressed by IL-5-induced lung eosinophilia. Thorax 2015; 70: 854–861.

Kirtland ME, Tsitoura DC, Durham SR, et al. Toll-like receptor agonists as adjuvants for allergen immunotherapy. Front Immunol 2020; 11: 599083.

Miller RL, Gerster JF, Owens ML, et al. Imiquimod applied topically: a novel immune response modifier and new class of drug. Int J Immunopharmacol 1999; 21: 1–14.

Tyring S. Immune response modification: imiquimod. Aust J Dermatol 1998; 39: Suppl. 1, S11–S13.

Goff PH, Hayashi T, Martinez-Gil L, et al. Synthetic Toll-like receptor 4 (TLR4) and TLR7 ligands as influenza virus vaccine adjuvants induce rapid, sustained, and broadly protective responses. J Virol 2015; 89: 3221–3235.

Das D, Sengupta I, Sarkar N, et al. Anti-hepatitis B virus (HBV) response of imiquimod based toll like receptor 7 ligand in HBV-positive human hepatocellular carcinoma cell line. BMC Infect Dis 2017; 17: 76.

McGowan D, Herschke F, Pauwels F, et al. Novel pyrimidine toll-like receptor 7 and 8 dual agonists to treat hepatitis B virus. J Med Chem 2016; 59: 7936–7949.

Lawitz E, Gruener D, Marbury T, et al. Safety, pharmacokinetics and pharmacodynamics of the oral toll-like receptor 7 agonist GS-9620 in treatment-naive patients with chronic hepatitis C. Antivir Ther 2015; 20: 699–708.

Gane EJ, Lim YS, Gordon SC, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J Hepatol 2015; 63: 320–328.

Landford RE, Guerra B, Chavez D, et al. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 2013; 144: 1508–1517.

Tran TD, Pryde DC, Jones P, et al. Design and optimisation of orally active TLR7 agonists for the treatment of hepatitis C virus infection. Bioorg Med Chem Lett 2011; 21: 2389–2393.

Fidock M, Souberbielle B, Laxton C, et al. The innate immune response, clinical outcomes, and ex vivo HCV antiviral efficacy of a TLR7 agonist (PF-4878691). Clin Pharmacol Ther 2011; 89: 821–829.

Rappe JCF, Finsterbusch K, Crotta S, et al. A TLR7 antagonist restricts interferon-dependent and -independent immunopathology in a mouse model of severe influenza. J Exp Med 2021; 218: e20201631.

Kan S, Hariyadi DM, Grainge C, et al. Airway epithelial-targeted nanoparticles for asthma therapy. Am J Physiol Lung Cell Mol Physiol 2020; 318: L500–L509.

Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000; 408: 740.

Chuang TH, Lee J, Kline L, et al. Toll-like receptor 9 mediates CpG-DNA signaling. J Leukoc Biol 2002; 71: 538–544.

Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 2002; 168: 4531–4537.

Kabelitz D. Expression and function of Toll-like receptors in T lymphocytes. Curr Opin Immunol 2007; 19: 39–45.
Bezemer GFG, Garssen J. TLR9 and COVID-19: a multidisciplinary theory of a multifaceted therapeutic target. *Front Pharmacol* 2021; 11: 601685.

Dasari V, Smith C, Schuessler A, et al. Induction of innate immune signatures following polyepitope protein-glycoprotein B-TLR4&9 agonist immunization generates multifunctional CMV-specific cellular and humoral immunity. *Hum Vaccines Immunother* 2014; 10: 1064–1077.

Moody MA, Santra S, Vandergrift NA, et al. Toll-like receptor 7/8 (TLR7/8) and TLR9 agonists cooperate to enhance HIV-1 envelope antibody responses in rhesus macaques. *J Virol* 2014; 88: 3329–3339.

Krieg AM, Efler SM, Wittpoth M, et al. Induction of systemic TH1-like innate immunity in normal volunteers following subcutaneous but not intravenous administration of CPG 7909, a synthetic B-class CpG oligodeoxynucleotide TLR9 agonist. *J Immunother* 2004; 27: 460–471.

McHutchison JG, Bacon BR, Gordon SC, et al. Phase 1B, randomized, double-blind, dose-escalation trial of CPG 10101 in patients with chronic hepatitis C virus. *Hepatology* 2007; 46: 1341–1349.

Vicari AP, Schmalbach T, Lekstrom-Himes J, et al. Safety, pharmacokinetics and immune effects in normal volunteers of CPG 10101 (ACTILON®), an investigational synthetic Toll-like receptor 9 agonist. *Antiviral Ther* 2007; 12: 741–751.

Winckelmann AA, Munk-Petersen LV, Rasmussen TA, et al. Administration of a toll-like receptor 9 agonist decreases the proviral reservoir in virologically suppressed HIV-infected patients. *PLoS ONE* 2013; 8: e62074.

Qin M, Li Y, Yang X, et al. Safety of Toll-like receptor 9 agonists: a systematic review and meta-analysis. *Immunopharmacol Immunotoxicol* 2014; 36: 251–260.

Manegold C, van Zendwijk N, Szczesna A, et al. A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Ann Oncol* 2012; 23: 72–77.

Machiels J-P, Kaminsky M-C, Keller U, et al. Phase Ib trial of the Toll-like receptor 9 agonist IMO-2055 in combination with 5-fluorouracil, cisplatin, and cetuximab as first-line palliative treatment in patients with recurrent/metastatic squamous cell carcinoma of the head and neck. *Invest New Drugs* 2013; 31: 1207–1216.

Smith DA, Conkling P, Richards DA, et al. Antitumor activity and safety of combination therapy with the Toll-like receptor 9 agonist IMO-2055, erlotinib, and bevacizumab in advanced or metastatic non-small cell lung cancer patients who have progressed following chemotherapy. *Cancer Immunol Immunother* 2014; 63: 787–796.

Hayden FG. Rhinovirus and the lower respiratory tract. *Rev Med Virol* 2004; 14: 17–31.

Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell* 2020; 182: 429–446.e14.

Corbett KS, Flynn B, Foulds KE, et al. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *N Engl J Med* 2020; 383: 1544–1555.

van Dooremalen N, Lambe T, Spencer A, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature* 2020; 586: 578–582.

Morris G, Berk M, Galecki P, et al. The neuro-immune pathophysiology of central and peripheral fatigue in systemic immune-inflammatory and neuro-immune diseases. *Mol Neurobiol* 2016; 53: 1195–1219.

Lucas K, Morris G, Anderson G, et al. The Toll-like receptor radical cycle pathway: a new drug target in immune-related chronic fatigue. *CNS Neurol Disord Drug Targets* 2015; 14: 838–854.

Loske J, Röhmel J, Lukassen S, et al. Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children. *Nat Biotechnol* 2020; 40: 316–324.

Muus C, Luecken MD, Eraslan G, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *bioRxiv* 2022; preprint [https://doi.org/10.1101/2020.04.19.049254]

Yoshida M, Worlock KB, Huang N, et al. Local and systemic responses to SARS-CoV-2 infection in children and adults. *Nature* 2021: 602: 321–327.

Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 2021; 21: 83–100.

Morikawa S, Kohdera U, Hosaka T, et al. Seasonal variations of respiratory viruses and etiology of human rhinovirus infection in children. *J Clin Virol* 2015; 73: 14–19.

Visseaux B, Burdet C, Voiriot G, et al. Prevalence of respiratory viruses among adults, by season, age, respiratory tract region and type of medical unit in Paris, France, from 2011 to 2016. *PLoS One* 2017; 12: e0180888.
Kim SY, Chang YJ, Cho HM, et al. Non-steroidal anti-inflammatory drugs for the common cold. *Cochrane Database Syst Rev* 2015; 8: CD006362.

Price WH. The isolation of a new virus associated with respiratory clinical disease in humans. *Proc Natl Acad Sci USA* 1956; 42: 892–896.

McLean GR. Developing a vaccine for human rhinoviruses. *J Vaccines Immun* 2014; 2: 16–20.

Doggett JE, Bynoe ML, Tyrrell DA. Some attempts to produce an experimental vaccine with rhinoviruses. *Br Med J* 1963; 1: 34–36.

Mitchison DA. Prevention of colds by vaccination against a rhinovirus: a report by the scientific committee on common cold vaccines. *Br Med J* 1965; 1: 1344–1349.

Douglas RG Jr, Couch RB. Parenteral inactivated rhinovirus vaccine: minimal protective effect. *Proc Soc Exp Biol Med* 1972; 139: 899–902.

Lee S, Nguyen MT, Currier MG, et al. A polyvalent inactivated rhinovirus vaccine is broadly immunogenic in rhesus macaques. *Nat Commun* 2016; 7: 12838.

Carrat F, Flahault A. Influenza vaccine: the challenge of antigenic drift. *Vaccine* 2007; 25: 6852–6862.

Medina RA, Garcia-Sastre A. Influenza A viruses: new research developments. *Nat Rev Microbiol* 2011; 9: 590–603.