Sendai Virus-Vectored Vaccines That Express Envelope Glycoproteins of Respiratory Viruses

Charles J. Russell * and Julia L. Hurwitz

Department of Infectious Diseases, St. Jude Children’s Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA; julia.hurwitz@stjude.org
* Correspondence: charles.russell@stjude.org; Tel.: +1-901-595-5648

Abstract: Human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), and human parainfluenza viruses (HPIVs) are leading causes of respiratory disease in young children, the elderly, and individuals of all ages with immunosuppression. Vaccination strategies against these pneumoviruses and paramyxoviruses are vast in number, yet no licensed vaccines are available. Here, we review development of Sendai virus (SeV), a versatile pediatric vaccine that can (a) serve as a Jennerian vaccine against HPIV1, (b) serve as a recombinant vaccine against HRSV, HPIV2, HPIV3, and HMPV, (c) accommodate foreign genes for viral glycoproteins in multiple intergenic positions, (d) induce durable, mucosal, B-cell, and T-cell immune responses without enhanced immunopathology, (e) protect cotton rats, African green monkeys, and chimpanzees from infection, and (f) be formulated into a vaccine cocktail. Clinical phase I safety trials of SeV have been completed in adults and 3–6-year-old children. Clinical testing of SeVRSV, an HRSV fusion (F) glycoprotein gene recombinant, has also been completed in adults. Positive results from these studies, and collaborative efforts with the National Institutes of Health and the Serum Institute of India assist advanced development of SeV-based vaccines. Prospects are now good for vaccine successes in infants and consequent protection against serious viral disease.

Keywords: vaccine vector; parainfluenza virus; paramyxovirus; pneumovirus; HRSV; envelope glycoprotein; fusion glycoprotein; attachment protein

1. The Clinical Need for Vaccines against Human Respiratory Syncytial Virus (HRSV), Human Metapneumovirus (HMPV) and Human Parainfluenza Virus (HPIV)

Acute respiratory tract infections are among the leading causes of death among children and adults worldwide [1]. The ongoing pandemic of coronavirus disease-19 (COVID-19) demonstrates the dire consequences of respiratory infections. Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the etiologic agent of COVID-19, was first identified in 2019, and has since caused more than 3 million deaths globally [2]. Influenza virus, another well-known respiratory pathogen, contributed to the deaths of more than 50 million people worldwide during the 1918 pandemic [3]. In 2008, seasonal influenza virus caused 28,000 to 111,500 deaths in children less than five years of age [4,5]. In a study by Gaunt et al. using a world health organization (WHO)-endorsed DALY modeling system, the disease burden of seasonal type A and type B influenza virus was respectively 6.8 and 1.7 per 1000 hospitalized children under the age of five [6].

Less well known are the human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), and human parainfluenza viruses (HPIV) types 1–4 [7–15]. Unfortunately, HRSV, HMPV, and the HPIVs cause considerable morbidity and mortality, particularly among young children and older adults, yet the disease consequences are often falsely attributed to influenza or ‘flu’ infections. In fact, the acute lower respiratory tract infections (ALRTI) caused by HRSV were estimated to kill 55,000–199,000 children under the age of five in 2005, and 94,600–149,400 children under the age of five in 2015 [8–13]. In the study described above by Gaunt et al., the disease burden for HRSV, HMPV, HPIV1,
HPIV2, and HPIV3 among hospitalized children under the age of five years was respectively 67.7, 4.0, 0.7, 0.3, and 4.4 per 1000 [6]. It is noteworthy that these estimates, totaling 77.1 per 1000 have far exceeded those for the influenza viruses. For patients who recover from any of the serious respiratory viral infections described above, residual damage to the airway can cause lifelong sequelae [16].

The immune system is capable of controlling infections with these viruses, as is demonstrated by the fact that children who have recovered from a first infection rarely become seriously ill when exposed to the same virus for a second time. Antibodies provide an important first line of defense against re-infection because they can block virus at the point of entry; T cells provide help to other effector cells as well as a fail-safe mechanism by killing infected cells if/when the virus evades the antibody response. Antibodies obtained from adults (pre-immune to HRSV) were once used routinely to passively protect infants from HRSV [17]. More recently, monoclonal antibodies are used to prevent serious infections with HRSV in vulnerable children [18–20]. While passive treatments can provide short-term protection, vaccines are needed to induce stable, endogenous virus-specific antibodies and T cells in young infants prior to a first virus exposure. Despite the passing of decades since the first candidate vaccines were developed, there remain no licensed vaccines for HRSV, HMPV, or the HPIVs.

2. Virology of the Paramyxoviruses and Pneumoviruses

2.1. Taxonomy and Phylogeny

The human parainfluenza viruses (HPIVs), and the related murine virus SeV, are members of the family Paramyxoviridae. HRSV and HMPV were also classified as paramyxoviruses until 2016 when the International Committee on the Taxonomy of Viruses (ICTV) reclassified these viruses as members of the newly created family Pneumoviridae [21,22]. The new species names for these viruses as defined by the 2016 reclassification are included in Tables 1 and 2. For historical purposes and consistency with the literature, this review will use the common names. Paramyxoviridae genus Respirovirus includes HPIV1 and HPIV3 as well as their closely related animal-virus counterparts SeV and bovine PIV3 (BPIV3), respectively (Figure 1), which have been developed as vaccine platforms. The genus Orthorubulavirus contains human pathogens HPIV2, HPIV4, and MuV in addition to the mammalian virus PIV5, another paramyxovirus vaccine vector [23,24]. Measles virus (MeV), one of the most infectious viruses and for which there has been a safe and effective vaccine for over fifty years, is a member of the genus Morbillivirus.

Table 1. Nomenclature of several viruses from Family Paramyxoviridae.

| Common Name                  | Species                     | Genus          |
|------------------------------|-----------------------------|----------------|
| Human parainfluenza virus 1  | Human respirovirus 1        | Respirovirus   |
| (HPIV1)                      | Human respirovirus 3        |                |
| Sentai virus (SeV)           | Marine respirovirus         |                |
| Human parainfluenza virus 3  | Human orthorubulavirus 2    | Orthorubulavirus|
| (HPIV3)                      | Human orthorubulavirus 4    |                |
| Parainfluenza virus 5 (PIV5) | Mammalian orthorubulavirus 5|                |
| Mumps virus (MuV)            | Mumps orthorubulavirus      |                |
| Canine distemper virus (CDV) | Canine morbillivirus        | Morbillivirus  |
| Measles virus (MeV)          | Measles morbillivirus       |                |
| Newcastle disease virus (NDV)| Avian avulavirus 1          | Orthoavulavirus|
| Hendra virus (HeV)           | Hendra henipavirus          | Henipavirus    |
| Nipah virus (NiV)            | Nipah henipavirus           |                |
Table 2. Nomenclature of several viruses from Family **Pneumoviridae**.

| Common Name                                      | Species               | Genus                |
|--------------------------------------------------|-----------------------|----------------------|
| Human metapneumovirus (HMPV)                     | Human metapneumovirus | Metapneumovirus      |
| Bovine respiratory syncytial virus (BRSV)        | Bovine orthopneumovirus | Orthopneumovirus     |
| Human respiratory syncytial virus (HRSV)         | Human orthopneumovirus | Orthopneumovirus     |

![Phylogenetic tree of F proteins](image)

The **Pneumoviridae** family contains HMPV from the genus **Metapneumovirus** and members of the genus **Orthopneumovirus**, including HRSV and its bovine counterpart BRSV. HRSV has been further divided into two subtypes, A and B. Paramyxoviruses and pneumoviruses are members of the order **Mononegavirales**, negative-strand RNA viruses with single stranded genomes. Other noteworthy members of this order are the families **Filoviridae** (Ebola and Marburg viruses), **Rhabdoviridae** (rabies virus and vesicular stomatitis virus, VSV), and **Bornaviridae** (Borna disease virus).

2.2. Genome and Virion Structure

The genomes of paramyxoviruses and pneumoviruses are single-stranded RNA that contain all genes in tandem (Figure 2). Genes common to these two virus families encode the nucleocapsid (N) protein that encapsidates genomic viral RNA, a polymerase-associated phosphoprotein (P), a matrix (M) protein that drives virus assembly, a fusion (F) surface glycoprotein that causes membrane fusion during viral entry, and a large (L) polymerase protein [25]. Attachment proteins are named hemagglutinin-neuraminidase (HN), hemagglutinin (H), or glycoprotein (G) depending on the functions of the protein.
and its associated virus. For some viruses, several other structural and non-structural genes may also be expressed including alternate genes from the P gene (V, C, I, W, and Y), a small hydrophobic protein (SH), and M2-1 and M2-2 genes. In general, paramyxoviruses are spherical with a diameter of 150–350 nm but can be pleomorphic or filamentous [25]. Pneumoviruses contain irregularly shaped spheres of 100–350 nm and filaments that are 60–200 nm in diameter and up to 10 µm in length [26,27]. Viral envelope glycoproteins project from a lipid bilayer envelope that is derived from the plasmid membrane of the host cell [28]. The SeV viral RNA, which contains 15,384 nucleotides (following the rule of six whereby the genome length is divisible by six), is bound by ~2600 N proteins, and together with 300 P and 50 L proteins, forms a helical nucleocapsid [29].

Figure 2. SeV replication cycle. (A) Genome structure of SeV. Polymerase complex genes nucleocapsid (N), phosphoprotein (P), and large polymerase (L) are color-coded red; matrix (M) is colored yellow; fusion (F) is colored blue; and hemagglutinin-neuraminidase (HN) is colored green. (B) Replication cycle of SeV. During step 1, HN binds sialic-acid-containing receptors, triggering irreversible conformational changes in the F protein that cause fusion of the viral envelope and host cell plasmid membrane. The genome and associated polymerase complexes are delivered into the cytoplasm, where they remain during replication. In step 2, the RNA-dependent RNA-polymerase transcribes viral genes serially starting from the 3′ end. In step 3, viral proteins are translated and processed. In steps 4 and 5, complementary genome is replicated and then serves as a template for replication of negative-sense genomes needed to produce progeny virions. In step 6, F and HN proteins traffic through the secretory pathway to the cell surface. The M protein associates with host cell proteins, viral ribonucleoproteins, envelope glycoprotein tails, and the plasmid membrane to help drive budding of progeny virions. HN receptor-destroying activity is needed for progeny virus release.

2.3. Replication Cycle

During viral entry, the receptor-binding protein binds receptors and triggers the F protein to undergo irreversible conformational changes that cause membrane fusion, allowing delivery of the genome and polymerase complex into the cytoplasm of the host cell [30–32]. Replication occurs in the cytoplasm (Figure 2). Early in infection, the viral RNA-dependent RNA-polymerase (vRNAP) transcribes mRNA starting with the N gene near the 3′ end of the genome and continuing serially to the L gene at the 5′ end. Intergenic junctions between genes encode a gene end that terminates transcription and synthesizes a poly-A tail, an intergenic sequence, and a gene start that caps the 5′ end of mRNA.
and initiates transcription (Figure 3). At each gene junction, the vRNAP may continue or terminate, resulting in a gradient of transcripts with N being most abundant and L the least abundant (Figure 4). The gene start sequences and their propensity to allow continuation of downstream transcription may differ, and this results in a substantial drop-off of transcription between the M and F genes of SeV [33]. Insertion of a foreign gene nearer the 3′ end of the genome causes a greater reduction in viral gene expression and a larger amount of attenuation than insertion of a foreign gene nearer the 5′ end [34,35]. After sufficient expression of viral proteins, the vRNAP replicates the entire genome to a complementary strand that serves as a template for negative-sense genome that will be incorporated into progeny virions (Figure 2). Assembly and budding are directed by the M protein, which interacts with viral nucleocapsids, the inner leaflet of the plasma membrane, glycoprotein cytoplasmic tails, and cellular factors [36–39].

**Figure 3.** Intergenic junctions of SeV. Upstream of each gene is a gene start sequence that directs initiation of transcription and capping of the 5′ end of the transcript. Downstream of each gene is a gene end sequence that directs termination of transcription and synthesis of a 3′ poly-A tail. A trinucleotide sequence separates gene end from gene start between the genes. Differences in gene start sequences modulate the level of polymerase continuation of transcription versus termination.

**Figure 4.** Differential transcription of SeV genes. Transcription begins at the 3′ end of the genome with the N gene. At each gene junction, the polymerase may continue to transcribe downstream genes or terminate transcription. Transcription start sequences vary between genes with the sequence upstream of the F gene resulting in the highest level of termination. As a result, the relative expression levels of F, HN, and L genes are substantially less than those of the upstream genes. Insertion of a foreign gene adds another gene junction and usually alters the ratio of gene transcripts in addition to decreasing the abundance of downstream transcripts. Positioning of foreign genes near the 3′ end of the genome causes greater virus attenuation than positioning foreign genes nearer the 5′ end.
2.4. Fusion (F) Glycoprotein Structures and Structural Intermediates

The paramyxoviruses and pneumoviruses contain an F surface glycoprotein that is a Type I integral membrane protein and a structural Class I viral fusion protein [40]. High-resolution structures have been obtained for ectodomains of the prefusion forms of the F proteins from PIV5 [41], HRSV [42], HMPV [43,44], and Hendra virus [45]. The presence of the transmembrane domain and cytoplasmic tails stabilizes the prefusion form of the protein [41,46], thus cell-surface expressed F protein is expected to adopt the native structure unless heated to supraphysiological temperatures [47].

While the F proteins from different viruses share little amino-acid sequence homology, their three-dimensional folding is similar. Cleavage into F1 and F2 subunits primes the protein by conversion into a fusion-capable pre-triggered form called the prefusion conformation [48]. The prefusion structure has a mushroom-like shape. The heptad repeat B (HRB) region, adjacent to the transmembrane domain, forms a triple-stranded coiled-coil stalk. Domains II and III form a bulbous head. The fusion peptide is bound in a cleft on the side of the head and its adjacent heptad repeat A (HRA) region forms four short alpha-helices and a beta-turn-beta structure. Upon activation by receptor binding, even at low temperatures that arrest complete refolding of the F protein, the HRB triple-stranded coiled coil from the prefusion conformation dissociates [31,32,41]. Next, a prehairpin intermediate forms in which HRA springs upward into a triple-stranded coiled coil that propels the fusion peptide into the target membrane [31,32,49,50]. Finally, HRB alpha-helices bind in an antiparallel orientation into the grooves formed by the HRA coiled coil, juxtaposing the adjacent fusion peptide and transmembrane domains and providing energy to do the work of membrane fusion [31,32]. Thus, the order of conformations adopted by the F protein are: (a) native prefusion, (b) a temperature-arrested intermediate with dissociated HRB regions, (c) a prehairpin intermediate with a triple-stranded HRA coiled coil, and (d) a postfusion six-helix bundle [31]. Post-fusion structures of the F protein ectodomain have been obtained for NDV [51,52], HPIV3 [46], and HRSV [53,54].

2.5. Attachment Protein Structures

The HN, H, and G proteins of the paramyxoviruses and pneumoviruses are Type II integral membrane proteins. High-resolution structures have been obtained for NDV HN [55–57], HPIV3 HN [58], MeV H [59–61], PIV5 HN [62], and NiV and HeV G [63–65]. The paramyxovirus HN, H, and G proteins form a dimer of dimers with a rod-shaped stalk and a globular head domain that has a six-bladed beta-propeller fold that is common to sialidases. Cocystal structures of neutralizing antibodies bound to the central conserved domain (CCD) of HRSV G have been obtained [66,67] but a high-resolution structure of the entire ectodomain has been elusive.

3. Past and Current Vaccine Candidates for HRSV, HMPV, and the HPIVs

Vaccine development for HRSV, HMPV, and the HPIVs has followed a long and difficult course. Among HRSV, HMPV, and the HPIVs, HRSV is the most frequent target of vaccine development because of the serious ALRTI caused by HRSV infections in human infants. In the 1960s a long-remembered study concerned a formalin-inactivated HRSV vaccine product. Unfortunately, the formalin treatment altered key epitopes on the virus surface during vaccine production, preventing the induction of neutralizing antibodies [68,69]. When vaccinated children were later naturally infected with HRSV, they fared worse than unvaccinated children. In fact, two vaccinated children died [70]. This outcome sent a shockwave through the scientific community. Since then, the fear of a repeat tragedy has hampered the clinical development of paramyxovirus and pneumovirus vaccines. The result is that six decades have passed with millions of additional lives lost due to paramyxovirus and pneumovirus infections and disease.

Since the 1960s, an astounding number of vaccines have been tested preclinically and some have progressed to clinical trials [71,72]. Strategies include the use of live-attenuated/chimeric vaccines, whole-inactivated vaccines, particle-based vaccines, subunit...
A number of protein-based vaccines have been tested, often with G or F proteins as their focus. The VRC, GSK, and Pfizer are each now testing HRSV F proteins, stabilized in prefusion forms (e.g., DS-Cav1) [42,44,79].
Particle-based vaccines have also been produced [80]. Mahdi et al. [81] performed a clinical study in pregnant women of a nanoparticle HRSV vaccine produced by Novavax. The particle was made using recombinant baculovirus that expresses the HRSV F protein. After the pregnant women gave birth, their children were monitored for ninety days for HRSV-associated, medically significant ALRTI. Infants born to vaccinated mothers experienced ALRTI reduced by 39.4% compared to placebo controls. There were mixed reviews when study data were released because the protocol’s primary endpoint was not met. Nonetheless, the results were viewed with optimism by some investigators because, for the first time, vaccination appeared to confer a degree of protection for infants against RSV.

Messenger RNA (mRNA) vaccines have come to the forefront of vaccine development in the SARS-CoV-2 field [82,83] and will assist the generation of multiple, new vaccine candidates for the paramyxovirus and pneumovirus fields [18]. Given the extraordinary effort dedicated to vaccine development for the paramyxoviruses and pneumoviruses, one can expect that new, licensed vaccine products are in sight.

4. Sendai Virus

SeV was discovered at Tohuko University Hospital in Sendai, Japan, in 1952 by Kuroya and colleagues after passage of a lung sample from a newborn child in mice [84]. At first, the virus was thought to be human-derived, but by 1954, Fukumi and colleagues realized that SeV was inadvertently isolated from mice [85,86]. SeV is currently recognized as a pathogen of mice, not humans, as SeV has never been known to cause human disease [87]. Outbreaks of SeV in mouse colonies worldwide have been reported including those displaying disease in mice (epizootic) [88] and others with long-term, unapparent transmission displaying little disease (enzootic) [89,90]. For enzootic strains, intranasal inoculation of large doses of SeV in large volumes that are directly aspirated into the lungs can result in substantial morbidity and mortality in susceptible strains of mice [91–94]. In contrast, contact transmission results in robust upper respiratory tract infection but limited infection and pathology in the lungs and no observable morbidity or mortality in healthy mice [92–94]. Short-range airborne transmission initiates in the nasopharynx or trachea and can remain local or disseminate yet also causes no observable morbidity or mortality [92]. Natural infection after transmission by enzootic strains causes no apparent morbidity in mice but provides robust immunity, even upon challenge with a high (e.g., greater than 1 million infectious units) amount of virus aspirated into the lungs [93].

5. Sendai Virus as a Vaccine Platform

5.1. Sendai Virus as a Jennerian Vaccine against HPIV1

In the late 1790s, clinician Edward Jenner inoculated a young boy with a substance from cow lesions, later discovered to contain cowpox virus. When Jenner subsequently exposed the boy to smallpox, the boy was protected. The live-virus vaccine, originally isolated from bovine and naturally attenuated in humans, was later shown to elicit virus-specific B cells, T helper cells, and cytotoxic T lymphocytes that persisted for decades after a single vaccination [95,96]. Approximately two centuries after development of the Jennerian smallpox vaccine, and after a global campaign led by the World Health Organization, the human population was declared free of smallpox virus by the World Health Assembly on 8 May 1980. The smallpox vaccine campaign has been the most successful vaccination effort in history in that it completely eradicated a human disease.

During the 1990s, researchers noted amino-acid sequence and antigenic similarities between HPIV1 and SeV [97,98]. These findings underpinned the development of numerous SeV-based respiratory virus vaccines (Table 3 provides a sampling of SeV research).
Table 3. Examples of research with SeV-based respiratory virus vaccines.

| Vaccine Name       | Inserted Antigen | Insertion Site | Host                                | Reference |
|--------------------|------------------|----------------|-------------------------------------|-----------|
| Sendai virus       | None             | None           | cotton rats                         | [99]      |
| Sendai virus       | None             | None           | African green monkeys (AGM)          | [100]     |
| Sendai virus       | None             | None           | AGM, chimpanzees                    | [101]     |
| Sendai virus       | None             | None           | human (adults/3–6 y.o.)              | [102,103] |
| rSV-RSV-G          | RSV A2 G         | F-HN           | cotton rats                         | [104,105] |
| rSV-RSV-F (SeVRSV)| RSV A2 F         | F-HN           | cotton rats                         | [105–108] |
| rSV-RSV-F (SeVRSV)| RSV A2 F         | F-HN           | AGM                                 | [109]     |
| rSV-RSV-F (SeVRSV)| RSV A2 F         | F-HN           | human adults                        | [103]     |
| rSV-RSV-Fs         | RSV A2 F (secreted) | F-HN         | cotton rats                         | [110]     |
| rSV-HPIV3-HN       | HPIV3 C243 HN    | F-HN           | cotton rats                         | [107,108] |
| rSV-HPIV3-F        | HPIV3 C243 F     | F-HN           | cotton rats                         | [107,111] |
| rSV-HPIV3-F(P-M)   | HPIV3 C243 F     | P-M            | cotton rats                         | [111]     |
| rSV-HPIV2-HN       | HPIV2 VR92 HN    | F-HN           | cotton rats                         | [108]     |
| rSV-HPIV2-F        | HPIV2 VR92 F     | F-HN           | cotton rats                         | [108]     |
| rSV-HMPV-Fs        | HMPV CAN00-16 F  | F-HN           | cotton rats                         | [112]     |

Because HPIV1 and SeV are closely related phylogenetically (Figure 1), experiments were first performed to develop SeV as a Jennerian vaccine against HPIV1. The approach was supported by findings that B cell, T helper cell, and cytotoxic T lymphocytes from human blood were all cross-reactive between HPIV1 and SeV [113,114]. In a proof of principle study, an intranasal inoculation of mice with HPIV1 was shown to elicit SeV-specific antibody responses and to protect mice from challenge with SeV [115]. As another example of cross-reactivity between HPIV1 and SeV, an intranasal inoculation of mice and cotton rats with SeV rapidly elicited durable immune responses in the respiratory mucosa and protection against challenges with HPIV1 [99,116]. Intranasal inoculation of SeV in African green monkeys induced high-magnitude, durable antibody responses against both SeV and HPIV1. In two separate laboratories, SeV conferred protection in non-human primates against challenge with HPIV1 [100,101].

Because SeV is delivered intranasally, long-lived antibody forming cells (AFCs) and T cells are established for residence in the respiratory tract. The AFCs underlie the epithelial cells that line the respiratory airway and can secrete virus-specific IgA. IgA antibodies are uniquely suited to transcytose epithelial cells, after which they can be tethered to airway cells, providing a first line of defense against incoming pathogens. The situation is unlike that for vaccines administered intramuscularly, which more-readily induce bone marrow-resident AFCs [99,117–119]. Live viral vaccines are also noted, as described above, for their induction of long-term immune responses in humans [95].

The target population of an SeV vaccine is the seronegative infant. Adults and seropositive children are not the target populations for SeV because they have pre-existing immune responses toward HPIV1 that cross-react with SeV. Their responses clear the SeV vaccine so quickly that the vaccine has only minimal influence on the immune response. Nonetheless, to ensure vaccine safety, Phase I clinical studies were first initiated in adults and then progressed to SeV-seropositive 3- to 6-year-old children [102,103]. In each age group, increasing vaccine doses were tested, including $5 \times 10^5$, $5 \times 10^6$, and $5 \times 10^7$ egg infectious doses-50 (EID$_{50}$). As expected, replication-competent virus was not observed in these participants. Despite their pre-existing immune responses, three of nine adult participants and eight of ten children exhibited a boost in virus-specific neutralizing antibodies [102,103]. Currently, SeV is being tested in SeV-seropositive, 1- to 2-year-old children. Resultant safety data will be used to support clinical studies in the vaccine’s target population, the seronegative infant.
5.2. Sendai Virus-Vectored HRSV Vaccine

Reverse genetics systems have been available for SeV since the mid-to-late 1990’s [120–122]. Researchers quickly established that SeV can accommodate foreign genes of 3 kb or more [34,35,123]. This raised the possibility of using SeV as a vaccine vector (Figure 6). SeV-vectored vaccines that have full-length or secreted forms of the HRSV F or G genes inserted between the F and HN genes of SeV were generated and evaluated in preclinical animal models (Table 3) [104,105,110]. Upon infection, the SeV-vectored HRSV F and G vaccines express unmodified, full-length envelope glycoproteins on the cell surface. HRSV F or G proteins expressed from SeV were shown to be excluded from progeny SeV-vector virions. The SeV-vectored HRSV vaccines elicited robust neutralizing antibody and T cell responses in cotton rats and protected from HRSV challenge. The HRSV F gene insertion was from the A2 strain and was able to generate neutralizing antibody responses and protect from challenge by both A and B subtypes in cotton rats [105,110]. The SeV-vectored HRSV vaccines did not trigger enhanced immunopathology after challenge [104,105,110] and were shown to be effective when inoculated into cotton rats that had maternal antibodies at titers comparable to those of a 2-month-old human infant [106].

Other vectored vaccines that express unmodified, full-length HRSV F express pre-fusion F (Pre-F) < postfusion F (Post-F), Pre-F > Post-F, or exclusively Pre-F [124]. In the context of transmembrane-anchorless, secreted F protein constructs containing stabilizing mutations, stabilized Pre-F has been shown to enhance production of neutralizing antibodies [124]. Similarly, stabilizing proline mutations have been inserted into coronavirus spike vaccines [125,126]. It is expected that natural protein conformations including Pre-F and Post-F are expressed upon SeV-infections of mammalian cells and that protein proportions will vary as a function of mammalian cell type and time post-infection.

The SeV vaccine designated SeVRSV, which contains the full-length HRSV A2 F gene inserted into the SeV F-HN gene junction, was advanced to a non-human primate study in African green monkeys [109]. As endorsed by the Food and Drug Administration (FDA), $1 \times 10^6$ EID$_{50}$ of the vaccine was administered both intranasally and intratracheally, and the African green monkeys were challenged with $1.4 \times 10^6$ PFU of the A2 strain of HRSV 28 days after vaccination. Vaccination stimulated production of HRSV-specific binding and neutralizing antibodies and protected against HRSV challenge without inducing immunopathology [109]. Subsequently, the SeV-vectored HRSV-F vaccine was advanced to human clinical trials in adults who were expected to be HRSV- and HPIV1/SeV-seropositive [127]. Again, as expected because of pre-existing immunity in adults, vaccine was quickly cleared. The recombinant vaccine genome was detected only transiently by PCR (tests of replication-competent SeVRSV were not performed). Furthermore, as expected, boosts of pre-existing immune responses were rare. Importantly, the vaccine was very well tolerated, inducing only mild to moderate reactions that were also observed in the placebo group. These results encourage progression of the SeV-vectored HRSV vaccine toward clinical studies in seronegative infants. Partnerships with the Serum Institute of India and the National Institutes of Health are ongoing to advance the SeVRSV vaccine through clinical trials.
5.3. Sendai Virus-Vectored HPIV3 Vaccine

SeV-vectored vaccines with the HPIV3 F or HN gene inserted between the F and HN genes of the SeV genome were generated and tested preclinically in cotton rats [107]. The vaccines were shown to express the inserted HPIV3 gene in infected Hep-2 cells. Vaccination of cotton rats resulted in the production of virus-specific binding antibodies, neutralizing antibodies, and interferon-gamma-producing T cells. Vaccination protected against the homotypic HPIV3 strain (C243) and an HPIV3 clinical isolate. Additional studies were completed on the HPIV3 F vaccine, varying the site of foreign antigen insertion (P-M or F-HN gene junction) or inoculated vaccine dose (200 or 2,000,000 PFU) [111]. While the P-M insertion caused mild attenuation in LLC-MK2 cells and cotton rats, it...
grew similarly to the F-HN construct in NHBE cells and yielded high levels of virus-specific neutralizing antibodies in cotton rat sera. Lower-dose vaccination resulted in only modest decreases in vaccine replication and serum antibody responses. All four vaccine combinations (two viruses and two doses) elicited complete protection from HPIV3 challenge in cotton rats, demonstrating the versatility of the SeV vaccine platform.

5.4. Sendai Virus-Vectored HPIV2 Vaccine and SeV-Vectored Vaccine Cocktails

Two SeV-vectored HPIV2 vaccines were generated by inserting the HPIV2 F or HN gene into the SeV F-HN gene junction [108]. Vaccination of cotton rats elicited serum neutralizing antibodies against the homotypic strain and heterotypic HPIV2 clinical isolates. Antibodies were durable for at least nine months after vaccination. The SeV-vectored vaccines also yielded complete protection from HPIV2 growth in the cotton rat lungs after challenge, even 9 and 11 months after vaccination. Simultaneous intranasal inoculation in cotton rats of a three-component cocktail of recombinant SeVs expressing HPIV2, HPIV3, and HRSV antigens yielded complete protection from challenge by HPIV1, HPIV2, HPIV3, and HRSV [108]. This suggests a cocktail of SeV-vectored vaccines could be administered early in childhood to target multiple respiratory pathogens.

5.5. Sendai Virus-Vectored HMPV Vaccine

An SeV-vectored HMPV was generated that had a truncated HMPV F gene inserted between the F and HN genes of the SeV genome [112]. As with the other SeV-vectored vaccines, intranasal vaccination of cotton rats elicited binding and neutralizing serum antibody responses and protected from challenge with the target virus, in this case HMPV [112,128].

6. Immunocompetence and the Vaccinated Host

A successful vaccine program depends on the immunocompetence of host populations. Even when vaccines prove clinically efficacious and advance to licensure, a subset of humans will not respond well. If an individual is receiving immunosuppressive drugs (e.g., for organ transplantation or treatments for allergy/autoimmune disease), it is understood that vaccine-induced immune responses may be sub-optimal. Less well recognized are the weak vaccine-induced immune responses among individuals who are vitamin-deficient or obese. Vitamin A deficiencies often go unnoticed in wealthy countries because they are presumed to affect the developing world exclusively. In fact, wealthy countries encompass low-income communities where there is little access to vitamin-rich foods [129] and frequent cases of vitamin deficiencies [130]. In children, vitamin A deficiencies/insufficiencies associate with poor immune responses toward vaccines [131]. Individuals with obesity, although apparently vitamin-replete based on blood tests, can suffer from low vitamin A levels in tissues including the lung [132] and from poor responses to vaccines [133,134].

In animal models of vitamin A deficiencies and obesity, supplementation with vitamin A at the time of vaccination improved immune responses toward vaccines [134–138]. In a clinical study, vitamin supplementation of children who had low baseline levels of vitamins A and D also significantly improved responses toward an influenza vaccine [131].

Supplementation programs have had positive outcomes in geographical areas where nutritional deficiencies are endemic [139]. However, because humans are a highly heterogeneous population, a one-size-fits-all vitamin supplementation program does not suffice [131]. This is due, in part, to the cross-regulation between vitamin A, a nuclear receptor ligand, and related ligands including vitamin D, sex hormones, and fat [140–146]. To assist the design of vitamin supplementation programs that may provide overall clinical benefit at the population level, a better understanding is needed of (i) baseline levels of nuclear receptor ligands in vaccine recipients and (ii) nuclear receptor ligand cross-regulatory capacities.
7. Outlook

Based on positive results from passive transfer studies, we know that immune effectors can protect humans from serious paramyxovirus and pneumovirus infections. Vaccines must now be advanced to ensure that virus-specific lymphocytes are activated in infants before a first virus exposure occurs. The SeV-based vaccines are attractive candidates for this purpose because they activate virus-specific lymphocytes and ensure long-term residence of immune cells in the respiratory tract. The COVID-19 pandemic has taught us that vaccine development can be rapid in response to an immediate need. Severe diseases caused by HRSV, HMPV, and HPIVs define another immediate need and encourage a call-to-action for rapid vaccine development. Other paramyxovirus vectors such as PIV5 and measles virus have been engineered to express glycoproteins from positive-strand viruses such as alphaviruses and coronaviruses [147–149]. SeV vaccines targeting positive-strand RNA viruses could also be produced and tested. Because of SeV’s cross-reactivity with HPIV1 and the immune responses generated in humans toward SeV by HPIV1 exposures, Sendai-vectored vaccines are best targeted to the pediatric arena. In this scenario, SeV-vectored vaccines have many attractive features, including high-level production in embryonated hen eggs or mammalian cell cultures, natural attenuation in humans, robust induction of mucosal and systemic B- and T-cell responses, and potent and durable immunogenicity. With combined efforts from government agencies, pharmaceutical companies, and research institutes, the outlook for an upcoming vaccine success is good.

Funding: This review article received no external funding.

Conflicts of Interest: J.L.H. has received funding from NIH/NIAID research grants R01AI088729 and P01 AI054955 for SeV vaccine development. C.J.R. has received NIH/NIAID research grant R01AI083370 in part to develop the SeV vector. The work completed in the studies and the views expressed here do not necessarily represent the official views of the National Institutes of Health. Both authors are inventors of patent US2014/0186397, Modified SeV vaccine and imaging vector (3 July 2014). Additional funding to both authors was from NCI P30 CA21765 and ALSAC.

References
1. Forum of international respiratory societies. In The Global Impact of Respiratory Disease, 2nd ed.; European Respiratory Society: Sheffield, UK, 2017. Available online: https://www.who.int (accessed on 3 March 2021).
2. Johns Hopkins University and Medicine Coronavirus Resource Center. Available online: https://coronavirus.jhu.edu (accessed on 6 March 2021).
3. Influenza 1918 Pandemic. Available online: https://www.cdc.gov (accessed on 1 May 2021).
4. Nair, H.; Brooks, W.A.; Katz, M.; Roca, A.; Berkley, J.A.; Madhi, S.A.; Zimmerman, J.M.; Gordon, A.; Sato, M.; Howie, S.; et al. Global burden of respiratory infections due to seasonal influenza in young children: A systematic review and meta-analysis. *Lancet* 2011, 378, 1917–1930. [CrossRef]
5. Iuliano, A.D.; Roguski, K.M.; Chang, H.H.; Muscatello, D.J.; Palekar, R.; Tempia, S.; Cohen, C.; Gran, J.M.; Schanzer, D.; Cowling, B.J.; et al. Global Seasonal Influenza-associated Mortality Collaborator, N., Estimates of global seasonal influenza-associated respiratory mortality: A modelling study. *Lancet* 2018, 391, 1285–1300. [CrossRef]
6. Gaunt, E.R.; Harvala, H.; McIntyre, C.; Templeton, K.E.; Simmonds, P. Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model. *J. Clin. Virol.* 2011, 52, 215–221. [CrossRef]
7. Edwards, K.M.; Zhu, Y.; Griffin, M.R.; Weinberg, G.A.; Hall, C.B.; Szilagyi, P.G.; Staat, M.A.; Ivane, M.; Prill, M.M.; Williams, J.V.; et al. Burden of human metapneumovirus infection in young children. *N. Engl. J. Med.* 2013, 368, 633–643. [CrossRef]
8. Shi, T.; McAllister, D.A.; O’Brien, K.L.; Simoes, E.A.F.; Madhi, S.A.; Gessner, B.D.; Polack, F.P.; Balsells, E.; Acacio, S.; Aguayo, C.; et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: A systematic review and modelling study. *Lancet* 2017, 390, 946–958. [CrossRef]
9. Respiratory Syncytial Virus (RSV). Available online: https://www.niaid.nih.gov (accessed on 3 March 2021).
10. Nair, H.; Nokes, D.J.; Gessner, B.D.; Dherani, M.; Madhi, S.A.; Singleton, R.J.; O’Brien, K.L.; Roca, A.; Wright, P.F.; Bruce, N.; et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: A systematic review and meta-analysis. *Lancet* 2010, 375, 1545–1555. [CrossRef]
37. Schmitt, A.P.; Leser, G.P.; Waning, D.L.; Lamb, R.A. Requirements for budding of paramyxovirus simian virus 5 virus-like particles. *J. Virol.* **2002**, *76*, 3952–3964. [CrossRef] [PubMed]
38. Takimoto, T.; Murti, K.G.; Bousse, T.; Scroggs, R.A.; Portner, A. Role of matrix and fusion proteins in budding of Sendai virus. *J. Virol.* **2001**, *75*, 11384–11391. [CrossRef] [PubMed]
39. Fouillot-Coriou, N.; Roux, L. Structure-function analysis of the Sendai virus F and HN cytoplasmic domain: Different role for the two proteins in the production of virus particle. *Virolology* **2000**, *270*, 464–475. [CrossRef] [PubMed]
40. Lamb, R.A.; Jardetzky, T.S. Structural basis of viral invasion: Lessons from paramyxovirus F. *Curr. Opin. Struct. Biol.* **2007**, *17*, 427–436. [CrossRef]
41. Yin, H.S.; Wen, X.; Paterson, R.G.; Lamb, R.A.; Jardetzky, T.S. Structure of the parainfluenza virus 5 F protein in its metastable, prefusion conformation. *Nature* **2006**, *439*, 38–44. [CrossRef] [PubMed]
42. McLellan, J.S.; Chen, M.; Leung, S.; Graepel, K.W.; Du, X.; Yang, Y.; Zhou, T.; Baxa, U.; Yasuda, E.; Beaumont, T.; et al. Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. *Science* **2013**, *340*, 1113–1117. [CrossRef] [PubMed]
43. Wen, X.; Krause, J.C.; Leser, G.P.; Cox, R.G.; Lamb, R.A.; Williams, J.V.; Crowe, J.E., Jr.; Jardetzky, T.S. Structure of the human metapneumovirus fusion protein with neutralizing antibody identifies a pneumovirus antigenic site. *Nat. Struct. Mol. Biol.* **2012**, *19*, 461–463. [CrossRef] [PubMed]
44. Battles, M.B.; Mas, V.; Olmedillas, E.; Cano, O.; Vazquez, M.; Rodriguez, L.; Melero, J.A.; McLellan, J.S. Structure and immunogenicity of pre-fusion-stabilized human metapneumovirus F glycoprotein. *Nat. Commun.* **2017**, *8*, 1528. [CrossRef]
45. Wong, J.J.; Paterson, R.G.; Lamb, R.A.; Jardetzky, T.S. Structure and stabilization of the Hendra virus F glycoprotein in its prefusion form. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 1056–1061. [CrossRef] [PubMed]
46. Yin, H.S.; Paterson, R.G.; Wen, X.; Lamb, R.A.; Jardetzky, T.S. Structure of the uncleaved ectodomain of the paramyxovirus (hPIV3) fusion protein. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9288–9293. [CrossRef] [PubMed]
47. Paterson, R.G.; Russell, C.J.; Lamb, R.A. Fusion protein of the paramyxovirus SV5: Destabilizing and stabilizing mutants of fusion activation. *Virolology* **2000**, *270*, 17–30. [CrossRef] [PubMed]
48. Bose, S.; Jardetzky, T.S.; Lamb, R.A. Timing is everything: Fine-tuned molecular machines orchestrate paramyxovirus entry. *Virolology* **2015**, *479*, 518–531. [CrossRef] [PubMed]
49. Baker, K.A.; Dutch, R.E.; Lamb, R.A.; Jardetzky, T.S. Structural basis for paramyxovirus-mediated membrane fusion. *Mol. Cell* **1999**, *3*, 309–319. [CrossRef] [PubMed]
50. Russell, C.J.; Jardetzky, T.S.; Lamb, R.A. Conserved glycine residues in the fusion peptide of the paramyxovirus fusion protein regulate activation of the native state. *J. Virol.* **2004**, *78*, 13727–13742. [CrossRef] [PubMed]
51. Swanson, K.; Wen, X.; Leser, G.P.; Paterson, R.G.; Lamb, R.A.; Jardetzky, T.S. Structure of the Newcastle disease virus F protein in the post-fusion conformation. *Virolology* **2010**, *402*, 372–379. [CrossRef] [PubMed]
52. Chen, L.; Gorman, J.J.; McKimm-Breschkin, J.; Lawrence, L.J.; Tulloch, P.A.; Smith, B.J.; Colman, P.M.; Lawrence, M.C. The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrand fusion. *Structure* **2001**, *9*, 255–266. [CrossRef] [PubMed]
53. McLellan, J.S.; Yang, Y.; Graham, B.S.; Kwong, P.D. Structure of respiratory syncytial virus fusion glycoprotein in the postfusion conformation reveals preservation of neutralizing epitopes. *J. Virol.* **2011**, *85*, 7788–7796. [CrossRef] [PubMed]
54. Swanson, K.A.; Settembre, E.C.; Shaw, C.A.; Dey, A.K.; Rappuoli, R.; Mandl, C.W.; Dormitzer, P.R.; Carfi, A. Structural basis for immunization with postfusion respiratory syncytial virus F glycoprotein (RSV F) to elicit high neutralizing antibody titers. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9619–9624. [CrossRef] [PubMed]
55. Crennell, S.; Takimoto, T.; Portner, A.; Taylor, G. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. *Nat. Struct. Biol.* **2000**, *7*, 1068–1074. [PubMed]
56. Yuan, P.; Paterson, R.G.; Leser, G.P.; Lamb, R.A.; Jardetzky, T.S. Structure of the ulcer strain newcastle disease virus hemagglutinin-neuraminidase reveals auto-inhibitory interactions associated with low virulence. *PLoS Pathog.* **2012**, *8*, e1002855. [CrossRef] [PubMed]
57. Yuan, P.; Swanson, K.A.; Leser, G.P.; Paterson, R.G.; Lamb, R.A.; Jardetzky, T.S. Structure of the Newcastle disease virus hemagglutinin-neuraminidase (HN) ectodomain reveals a four-helix bundle stalk. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14920–14925. [CrossRef] [PubMed]
58. Lawrence, M.C.; Borg, N.A.; Streltsov, V.A.; Pilling, P.A.; Epa, V.C.; Varghese, J.N.; McKimm-Breschkin, J.L.; Colman, P.M. Structure of the haemagglutinin-neuraminidase from human parainfluenza virus type III. *J. Mol. Biol.* **2004**, *335*, 1343–1357. [CrossRef] [PubMed]
59. Colf, L.A.; Juo, Z.S.; Garcia, K.C. Structure of the measles virus hemagglutinin. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1227–1228. [CrossRef] [PubMed]
60. Hashiguchi, T.; Kajikawa, M.; Maita, N.; Takeda, M.; Kuroki, K.; Sasaki, K.; Kohda, D.; Yanagi, Y.; Maenaka, K. Crystal structure of measles virus hemagglutinin provides insight into effective vaccines. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19535–19540. [CrossRef] [PubMed]
61. Hashiguchi, T.; Ose, T.; Kubota, M.; Maita, N.; Kamishikiryo, J.; Maenaka, K.; Yanagi, Y. Structure of the measles virus hemagglutinin bound to its cellular receptor SLAM. *Nat. Struct. Mol. Biol.* **2011**, *18*, 135–141. [CrossRef] [PubMed]
62. Yuan, P.; Thompson, T.B.; Wurzburg, B.A.; Paterson, R.G.; Lamb, R.A.; Jardetzky, T.S. Structural studies of the parainfluenza virus 5 hemagglutinin-neuraminidase tetramer in complex with its receptor, sialyllactose. *Structure* **2005**, *13*, 803–815. [CrossRef] [PubMed]
63. Xu, K.; Rajashankar, K.R.; Chan, Y.P.; Himanen, J.P.; Broder, C.C.; Nikolov, D.B. Host cell recognition by the henipaviruses: Crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. Proc. Natl. Acad. Sci. USA 2008, 105, 9953–9958. [CrossRef] [PubMed]

64. Bowden, T.A.; Aricescu, A.R.; Gilbert, R.J.; Grimes, J.M.; Jones, E.Y.; Stuart, D.I. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. Nat. Struct. Mol. Biol. 2008, 15, 567–572. [CrossRef]

65. Bowden, T.A.; Crispin, M.; Harvey, D.J.; Jones, E.Y.; Stuart, D.I. Dimeric architecture of the Hendra virus attachment glycoprotein: Evidence for a conserved mode of assembly. J. Virol. 2010, 84, 6208–6217. [CrossRef]

66. Fedechkin, S.O.; George, N.L.; Wolff, J.T.; Kauvar, L.M.; DuBois, R.M. Structures of respiratory syncytial virus G antigen bound to broadly neutralizing antibodies. Sci. Immunol. 2018, 3. [CrossRef]

67. Fedechkin, S.O.; George, N.L.; Nunez Castrejon, A.M.; Dillen, J.R.; Kauvar, L.M.; DuBois, R.M. Conformational Flexibility in Respiratory Syncytial Virus G Neutralizing Epitopes. J. Virol. 2020, 94, e01879-19. [CrossRef] [PubMed]

68. Murphy, B.R.; Walsh, E.E. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. J. Clin. Microbiol. 1988, 26, 1595–1597. [CrossRef] [PubMed]

69. Murphy, B.R.; Prince, G.A.; Walsh, E.E.; Kim, H.W.; Parrott, R.H.; Hemming, V.G.; Rodriguez, W.J.; Chanock, R.M. Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. J. Clin. Microbiol. 1986, 24, 197–202. [CrossRef]

70. Fulginiti, V.A.; Eller, J.J.; Sieber, O.F.; Joyner, J.W.; Minamitani, M.; Meiklejohn, G. Respiratory virus immunization I. A field trial of two inactivated respiratory virus vaccines; an aqeous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. Am. J. Epidemiol. 1969, 89, 435–448. [CrossRef]

71. Biagi, C.; Dondi, A.; Scarppini, S.; Rocca, A.; Vandini, S.; Poletti, G.; Lanari, M. Current State and Challenges in Developing Respiratory Syncytial Virus Vaccines. Vaccines 2020, 8, 672. [CrossRef] [PubMed]

72. Killikelly, A.; Tunis, M.; House, A.; Quach, C.; Vaudry, W.; Moore, D. Overview of the respiratory syncytial virus vaccine candidate pipeline in Canada. Can. Commun. Dis. Rep. 2020, 46, 56–61. [CrossRef] [PubMed]

73. Kim, Y.I.; DeVincenzo, J.P.; Jones, B.G.; Rudraraju, R.; Harrison, L.; Meyers, R.; Cehelsky, J.; Alvarez, J.; Hurwitz, J.L. Respiratory syncytial virus human experimental infection model: Provenance, production, and sequence of low-passaged memphis-37 challenge virus. PLoS ONE 2014, 9, e131100. [CrossRef]

74. RSV Clinical Trial Tracker. Available online: https://www.resources/and-mab-trial-tracker (accessed on 1 May 2021).

75. Schickli, J.H.; Kaur, J.; Tang, R.S. Nonclinical phenotypic and genotypic analyses of a Phase 1 pediatric respiratory syncytial virus vaccine candidate MEDI-559 (rA2cp248/404/1030DeltaSH) at permissive and non-permissive temperatures. Virus Res. 2012, 169, 38–47. [CrossRef] [PubMed]

76. Mok, H.; Tollefson, S.J.; Podsiad, A.B.; Shepherd, B.E.; Polosukhin, V.V.; Johnston, R.E.; Williams, J.V.; Crowe, J.E., Jr. An alfaphavirus replicon-based human metapneumovirus vaccine is immunogenic and protective in mice and cotton rats. J. Virol. 2008, 82, 11410–11418. [CrossRef]

77. Skiadopoulos, M.H.; Schmidt, A.C.; Riggs, J.M.; Surman, S.R.; Elkins, W.R.; St Claire, M.; Collins, P.L.; Murphy, B.R. Determinants of the host range restriction of replication of bovine parainfluenza virus type 3 in rhesus monkeys are polygenic. J. Virol. 2003, 77, 1141–1148. [CrossRef]

78. Karron, R.A.; Thumar, B.; Schappell, E.; Surman, S.; Murphy, B.R.; Collins, P.L.; Schmidt, A.C. Evaluation of two chimeric bovine-human parainfluenza virus type 3 vaccines in infants and young children. Vaccine 2012, 30, 3975–3981. [CrossRef]

79. Crank, M.C.; Ruckwardt, T.J.; Chen, M.; Morabito, K.M.; Phung, E.; Costner, P.J.; Holman, L.A.; Hickman, S.P.; Berkowitz, N.M.; Gordon, I.J.; et al. A proof of concept for structure-based vaccine design targeting RSV in humans. Science 2019, 365, 505–509. [CrossRef]

80. Kim, K.H.; Lee, Y.T.; Hwang, H.S.; Kwon, Y.M.; Kim, M.C.; Ko, E.J.; Lee, J.S.; Lee, Y.; Kang, S.M. Virus-Like Particle Vaccine Containing the F Protein of Respiratory Syncytial Virus Confers Protection without Pulmonary Disease by Modulating Specific Subsets of Dendritic Cells and Effecting T Cells. J. Virol. 2015, 89, 11692–11705. [CrossRef] [PubMed]

81. Madhi, S.A.; Polack, F.P.; Piedra, P.A.; Munoz, F.M.; Trenholme, A.A.; Simes, E.A.F.; Swaney, G.K.; Agrawal, S.; Ahmed, K.; August, A.; et al. Respiratory Syncytial Virus Vaccine during Pregnancy and Effects in Infants. N. Engl. J. Med. 2020, 383, 426–439. [CrossRef]

82. Polack, F.P.; Thomas, S.J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J.L.; Perez Marc, G.; Moreira, E.D.; Zerbini, C.; et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N. Engl. J. Med. 2020, 383, 2603–2615. [CrossRef]

83. Widge, A.T.; Rouphael, N.G.; Jackson, L.A.; Anderson, E.J.; Roberts, P.C.; Makhen, M.; Chappell, J.D.; Denison, M.R.; Stevens, L.J.; Prijipisters, A.J.; et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. N. Engl. J. Med. 2021, 384, 80–82. [CrossRef] [PubMed]

84. Kuroya, M.; Ishida, N. Newborn virus pneumonitis (type Sendai). II. The isolation of a new virus possessing hemagglutinin activity. Yokohama Med. Bull. 1953, 4, 217–233. [PubMed]

85. Fukushima, H.; Nishikawa, F.; Kitayama, T. A pneumotropic virus from mice causing hemagglutination. Jpn. J. Med. Sci. Biol. 1954, 7, 345–363. [CrossRef]

86. Ishida, N.; Homma, M. Sendai virus. Adv. Virus Res. 1978, 23, 349–383. [PubMed]

87. Karron, R.A.; Collins, P.L. Parainfluenza viruses. In Fields Virology, 5th ed.; Knipe, D.M., Howley, P.M., Griffin, D.E., Martin, M.A., Lamb, R.A., Roizman, B., Straus, S.E., Eds.; Lippincott Williams and Wilkins: Philadelphia, PA, USA, 2007; pp. 1497–1526.
88. Bhatt, P.N.; Jonas, A.M. An epizootic of Sendai infection with mortality in a barrier-maintained mouse colony. *Am. J. Epidemiol.* 1974, 100, 222–229. [CrossRef] [PubMed]

89. Parker, J.C.; Tennant, R.W.; Ward, T.G.; Rowe, W.P. Enzootic Sendai Virus Infections in Mouse Breeder Colonies within the United States. *Science* 1964, 146, 936–938. [CrossRef] [PubMed]

90. Profeta, M.L.; Lief, F.S.; Plotkin, S.A. Enzootic sendai infection in laboratory hamsters. *Am. J. Epidemiol.* 1969, 89, 316–324. [CrossRef]

91. Faisca, P.; Anh, D.B.; Desmecht, D.J. Sendai virus-induced alterations in lung structure/function correlate with viral loads and reveal a wide resistance/susceptibility spectrum among mouse strains. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2005, 289, L777–L787. [CrossRef] [PubMed]

92. Burke, C.W.; Bridges, O.; Brown, S.; Rahija, R.; Russell, C.J. Mode of parainfluenza virus transmission determines the dynamics of primary infection and protection from reinfection. *PLoS Pathog.* 2013, 9, e1003786. [CrossRef] [PubMed]

93. Burke, C.W.; Li, M.; Hurwitz, J.L.; Vogel, P.; Russell, C.J. Relationships among dissemination of primary parainfluenza virus infection in the respiratory tract, mucosal and peripheral immune responses, and protection from reinfection: A noninvasive bioluminescence-imaging study. *J. Virol.* 2015, 89, 3568–3583. [CrossRef]

94. Burke, C.W.; Mason, J.N.; Surman, S.L.; Jones, B.G.; Dalloneau, E.; Hurwitz, J.L.; Russell, C.J. Illumination of parainfluenza virus infection and transmission in living animals reveals a tissue-specific dichotomy. *PLoS Pathog.* 2011, 7, e1002134. [CrossRef]

95. Crotty, S.; Felgner, P.; Davies, H.; Glidewell, J.; Villarreal, L.; Ahmed, R. Cutting edge: Long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* 2003, 171, 4969–4973. [CrossRef]

96. Amanna, I.J.; Slifka, M.K.; Crotty, S. Immunity and immunological memory following smallpox vaccination. *Immunol. Rev.* 2006, 211, 320–337. [CrossRef] [PubMed]

97. Gorman, W.L.; Gill, D.S.; Scroggs, R.A.; Portner, A. The hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus type 1 and Sendai virus have high structure-function similarity with limited antigenic cross-reactivity. *Virology* 1990, 175, 211–221. [CrossRef]

98. Lyn, D.; Gill, D.S.; Scroggs, R.A.; Portner, A. The nucleoproteins of human parainfluenza virus type 1 and Sendai virus share amino acid sequences and antigenic and structural determinants. *J. Gen. Virol.* 1991, 72, 983–987. [CrossRef]

99. Sealy, R.; Jones, B.G.; Surman, S.L.; Hurwitz, J.L. Robust IgA and IgG-producing antibody forming cells in the diffuse-NALT and lungs of Sendai virus-vaccinated cotton rats associate with rapid protection against human parainfluenza virus type 1. *Vaccine* 2010, 28, 6749–6756. [CrossRef] [PubMed]

100. Hurwitz, J.L.; Soike, K.F.; Sangster, M.Y.; Portner, A.; Sealy, R.E.; Dawson, D.H.; Coleclough, C. Intranasal Sendai virus vaccine protects African green monkeys from infection with human parainfluenza virus-type 1. *Vaccine* 1997, 15, 533–540. [CrossRef]

101. Skiadopoulos, M.H.; Surman, S.R.; Riggs, J.M.; Elkins, W.R.; St Claire, M.; Nishio, M.; Garcin, D.; Kolakofsky, D.; Collins, P.L.; Murphy, B.R. Sendai virus, a murine parainfluenza virus type 1, replicates to a level similar to human PIV1 in the upper and lower respiratory tract of African green monkeys and chimpanzees. *Virology* 2002, 297, 153–160. [CrossRef] [PubMed]

102. Slobod, K.S.; Shenep, J.L.; Lujan-Zilbermann, J.; Allison, K.; Brown, B.; Scroggs, R.A.; Portner, A.; Coleclough, C.; Hurwitz, J.L. Safety and immunogenicity of intranasal murine parainfluenza virus type 1 (Sendai virus) in healthy human adults. *Vaccine* 2004, 22, 3182–3186. [CrossRef] [PubMed]

103. Adderson, E.; Branum, K.; Sealy, R.E.; Jones, B.G.; Surman, S.L.; Penkert, R.; Freiden, P.; Slobod, K.S.; Gaur, A.H.; Hayden, R.T.; et al. Safety and immunogenicity of an intranasal Sendai virus-based human parainfluenza virus type 1 vaccine in 3- to 6-year-old children. *Clin. Vaccine Immunol.* 2015, 22, 298–303. [CrossRef]

104. Takimoto, T.; Hurwitz, J.L.; Coleclough, C.; Prouser, C.; Krishnamurthy, S.; Zhan, X.; Boyd, K.; Scroggs, R.A.; Brown, B.; Nagai, Y.; et al. Recombinant Sendai virus expressing the G glycoprotein of respiratory syncytial virus (RSV) elicits immune protection against RSV. *J. Virol.* 2004, 78, 6043–6047. [CrossRef] [PubMed]

105. Zhan, X.; Hurwitz, J.L.; Krishnamurthy, S.; Takimoto, T.; Boyd, K.; Scroggs, R.A.; Surman, S.; Portner, A.; Slobod, K.S. Respiratory syncytial virus (RSV) fusion protein expressed by recombinant Sendai virus elicits B-cell and T-cell responses in cotton rats and confers protection against RSV and NS1. *Vaccine* 2007, 25, 8782–8793. [CrossRef]

106. Jones, B.G.; Sealy, R.E.; Surman, S.L.; Portner, A.; Russell, C.J.; Slobod, K.S.; Dornmitzer, P.R.; DeVincenzo, J.; Hurwitz, J.L. Sendai virus-based RSV vaccine protects against RSV challenge in an in vivo maternal antibody model. *Vaccine* 2014, 32, 3264–3273. [CrossRef] [PubMed]

107. Zhan, X.; Slobod, K.S.; Krishnamurthy, S.; Luque, L.E.; Takimoto, T.; Jones, B.; Surman, S.; Russell, C.J.; Portner, A.; Hurwitz, J.L. Sendai virus recombinant vaccine expressing hPIV-3 HN or F elicits protective immunity and combines with a second recombinant to prevent hPIV-1, hPIV-3 and RSV infections. *Vaccine* 2008, 26, 3480–3488. [CrossRef] [PubMed]

108. Jones, B.; Zhan, X.; Mishin, V.; Slobod, K.S.; Surman, S.; Russell, C.J.; Portner, A.; Hurwitz, J.L. Human PIV-2 recombinant Sendai virus (rSeV) elicits durable immunity and combines with two additional rSeVs to protect against hPIV-1, hPIV-2, hPIV-3, and RSV. *Vaccine* 2009, 27, 1848–1857. [CrossRef]

109. Jones, B.G.; Sealy, R.E.; Rudraraju, R.; Traina-Dorge, V.L.; Finneyfrock, B.; Cook, A.; Takimoto, T.; Portner, A.; Hurwitz, J.L. Sendai virus-based RSV vaccine protects African green monkeys from RSV infection. *Vaccine* 2012, 30, 959–968. [CrossRef]

110. Zhan, X.; Slobod, K.S.; Jones, B.G.; Sealy, R.E.; Takimoto, T.; Boyd, K.; Surman, S.; Russell, C.J.; Portner, A.; Hurwitz, J.L. Sendai virus recombinant vaccine expressing a secreted, unconstrained respiratory syncytial virus fusion protein protects against RSV in cotton rats. *Int. Immunol.* 2015, 27, 229–236. [CrossRef] [PubMed]
111. Mason, J.N.; Elbahesh, H.; Russell, C.J. Influence of antigen insertion site and vector dose on immunogenicity and protective capacity in Sendai virus-based human parainfluenza virus type 3 vaccines. *J. Virol.* 2013, 87, 5995–5969. [CrossRef]

112. Russell, C.J.; Jones, B.G.; Sealy, R.E.; Surman, S.L.; Mason, J.N.; Hayden, R.T.; Tripp, R.A.; Takimoto, T.; Hurwitz, J.L. A Sendai virus recombinant vaccine expressing a gene for truncated human metapneumovirus (hMPV) fusion protein protects cotton rats from hMPV challenge. *Virology* 2017, 508, 60–66. [CrossRef]

113. Dave, V.P.; Allan, J.E.; Slobod, K.S.; Smith, F.S.; Ryan, K.W.; Takimoto, T.; Power, U.F.; Portner, A.; Hurwitz, J.L. Viral cross-reactivity and antigenic determinants are recognized by human parainfluenza virus type 1-specific cytotoxic T-cells. *Virology* 1994, 199, 376–383. [CrossRef]

114. Smith, F.S.; Portner, A.; Leggiadro, R.J.; Turner, E.V.; Hurwitz, J.L. Age-related development of human memory T-helper and B-cell responses toward parainfluenza virus type-1. *Virology* 1994, 205, 453–461. [CrossRef]

115. Sangster, M.; Smith, F.S.; Coleclough, C.; Hurwitz, J.L. Human parainfluenza virus type 1 immunization of infant mice protects from subsequent Sendai virus infection. *Virology* 1995, 212, 13–19. [CrossRef]

116. Rudraraju, R.; Surman, S.; Jones, B.; Sealy, R.; Woodland, D.L.; Hurwitz, J.L. Phenotypes and functions of persistent Sendai virus-induced antibody forming cells and CD8+ T cells in diffuse nasal-associated lymphoid tissue tipify lymphocyte responses of the gut. *Virology* 2011, 410, 429–436. [CrossRef] [PubMed]

117. Sealy, R.E.; Surman, S.L.; Vogel, P.; Hurwitz, J.L. Antibody-secreting cells in respiratory tract tissues in the absence of eosinophils as supportive partners. *Int. Immunol.* 2016, 28, 559–564. [CrossRef]

118. Sealy, R.; Webby, R.J.; Crumpton, J.C.; Hurwitz, J.L. Differential localization and function of antibody-forming cells responsive to inactivated or live-attenuated influenza virus. *Int. Immunol.* 2015, 25, 183–195. [CrossRef] [PubMed]

119. Hyland, L.; Sangster, M.; Sealy, R.; Coleclough, C. Respiratory virus infection of mice provokes a permanent humoral immune response. *J. Virol.* 1994, 68, 6083–6086. [CrossRef] [PubMed]

120. Garcin, D.; Pelet, T.; Calain, P.; Roux, L.; Curran, J.; Kolakofsky, D. A highly recombinogenic system for the recovery of infectious Sendai paramyxovirus from cDNA: Generation of a novel copy-back nondefective interfering virus. *Science* 1995, 269, 221–226. [CrossRef]

121. Kato, A.; Sakai, Y.; Shioda, T.; Kondo, T.; Nakanishi, M.; Nagai, Y. Initiation of Sendai virus multiplication from transfected cDNA. *FEBS Lett.* 1998, 438, 376–383. [CrossRef] [PubMed]

122. Mazur, N.I.; Higgins, D.; Nunes, M.C.; Melero, J.A.; Langedijk, A.C.; Horsley, N.; Buchholz, U.J.; Openshaw, P.J.; McLellan, J.S.; Englund, J.A.; et al. Respiratory Syncytial Virus Network. The respiratory syncytial virus vaccine landscape: Lessons from the graveyard and promising candidates. *Lancet Infect. Dis.* 2018, 18, e295–e311. [CrossRef]

123. Pallesen, J.; Wang, N.; Corbett, K.S.; Wrapp, D.; Kirchdoerfer, R.N.; Turner, H.L.; Cottrell, C.A.; Becker, M.M.; Wang, L.; Shi, W.; et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc. Natl. Acad. Sci. USA* 2015, 112, 15058–15074. [CrossRef]

124. Beck, M.A. Increased risk of influenza among vaccinated adults who are obese. *Int. J. Obes.* 2019, 43, 1324–1330. [CrossRef] [PubMed]

125. Scaggs Huang, F.; Bernstein, D.I.; Slobod, K.S.; Mason, J.N.; Hayden, R.T.; Tripp, R.A.; Takimoto, T.; Hurwitz, J.L. A Sendai virus recombinant vaccine expressing a gene for truncated human metapneumovirus (hMPV) fusion protein protects cotton rats from hMPV challenge. *Virology* 2017, 508, 60–66. [CrossRef]

126. Wrapp, D.; Wang, N.; Corbett, K.S.; Goldsmith, J.A.; Hsieh, C.L.; Abiona, O.; Graham, B.S.; McLellan, J.S. Cryo-EM structure of SARS-CoV-2 at 2.4Å resolution. *Science* 2020, 367, 1260–1263. [CrossRef] [PubMed]

127. Pallesen, J.; Wang, N.; Corbett, K.S.; Goldsmith, J.A.; Hsieh, C.L.; Abiona, O.; Graham, B.S.; McLellan, J.S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020, 367, 1260–1263. [CrossRef]

128. Russell, C.J.; Jones, B.G.; Sealy, R.E.; Surman, S.L.; Mason, J.N.; Hayden, R.T.; Tripp, R.A.; Takimoto, T.; Hurwitz, J.L. Retinol binding protein and vitamin D associations with serum antibody isotypes, serum influenza virus-specific neutralizing activities and airway cytokine profiles. *Clin. Exp. Immunol.* 2016, 183, 239–247. [CrossRef]

129. Patel, N.; Penkert, R.R.; Jones, B.G.; Sealy, R.E.; Surman, S.L.; Sun, Y.; Tang, L.; DeBeauchamp, J.; Webb, A.; Richardson, J.; et al. Baseline Serum Vitamin A and D Levels Determine Benefit of Oral Vitamin A&D Supplements to Humoral Immune Responses Following Pediatric Influenza Vaccination. *Int. J. Env. Res. Public Health* 2021, 18, 554–559. [CrossRef]

130. Mason, J.N.; Elbahesh, H.; Russell, C.J.; Hayden, R.T.; Tripp, R.A.; Takimoto, T.; Hurwitz, J.L. Human Metapneumovirus: A Largely Unrecognized Threat to Human Health. *Microbiol. Immunol.* 1996, 40, 221–226. [CrossRef]

131. Patel, N.; Penkert, R.R.; Jones, B.G.; Sealy, R.; Woodland, D.L.; Hurwitz, J.L. Phenotypes and functions of persistent Sendai virus-induced antibody forming cells and CD8+ T cells in diffuse nasal-associated lymphoid tissue tipify lymphocyte responses of the gut. *Virology* 2011, 410, 429–436. [CrossRef] [PubMed]

132. Mason, J.N.; Elbahesh, H.; Russell, C.J.; Hayden, R.T.; Tripp, R.A.; Takimoto, T.; Hurwitz, J.L. Human Metapneumovirus: A Largely Unrecognized Threat to Human Health. *Microbiol. Immunol.* 1996, 40, 221–226. [CrossRef]

133. Neidich, S.D.; Green, W.D.; Rebeles, J.; Karlsson, E.A.; Schultz-Cherry, S.; Noah, T.L.; Chakladar, S.; Hudgens, M.G.; Weir, S.S.; Beck, M.A. Increased risk of influenza among vaccinated adults who are obese. *Int. J. Obes.* 2017, 41, 1324–1330. [CrossRef] [PubMed]
134. Penkert, R.R.; Cortez, V.; Karlsson, E.A.; Livingston, B.; Surman, S.L.; Li, Y.; Catharine Ross, A.; Schultz-Cherry, S.; Hurwitz, J.L. Vitamin A Corrects Tissue Deficits in Diet-Induced Obese Mice and Reduces Influenza Infection After Vaccination and Challenge. *Obesity* 2020, 28, 1631–1636. [CrossRef] [PubMed]

135. Penkert, R.R.; Rowe, H.M.; Surman, S.L.; Sealy, R.E.; Vogel, P.; Neale, G.; Hurwitz, J.L. Vitamin A deficient mice exhibit increased viral antigens and enhanced cytokine/chemokine production in nasal tissues following respiratory virus infection despite the presence of FoxP3+ T cells. *Int. Immunol.* 2016, 28, 139–152. [CrossRef] [PubMed]

136. Penkert, R.R.; Surman, S.L.; Jones, B.G.; Rudraraju, R.; Sealy, R.E.; Hurwitz, J.L. Intranasal administration of retinyl palmitate with a respiratory virus vaccine corrects impaired mucosal IgA response in the vitamin A deficient host. *Clin. Vaccine Immunol.* 2014, 21, 598–601. [CrossRef] [PubMed]

137. Surman, S.L.; Jones, B.G.; Rudraraju, R.; Sealy, R.E.; Hurwitz, J.L. Oral retinyl palmitate or retinoic acid corrects mucosal IgA responses toward an intranasal influenza virus vaccine in vitamin A deficient mice. *Vaccine* 2014, 32, 2521–2524. [CrossRef]

138. Sommer, A. Vitamin A, infectious disease, and childhood mortality: A 2 cent solution? *J. Infect. Dis.* 1993, 167, 1003–1007. [CrossRef]

139. Krycer, J.R.; Brown, A.J. Cross-talk between the androgen receptor and the liver X receptor: Implications for cholesterol homeostasis. *J. Biol. Chem.* 2011, 286, 20637–20647. [CrossRef] [PubMed]

140. Evans, R.M.; Mangelsdorf, D.J. Nuclear Receptors, RXR, and the Big Bang. *Cell* 2014, 157, 255–266. [CrossRef] [PubMed]

141. Jones, B.G.; Sealy, R.E.; Penkert, R.R.; Surman, S.L.; Birshtein, B.K.; Xu, B.; Neale, G.; Maul, R.W.; Gearhart, P.J.; Hurwitz, J.L. From Influenza Virus Infections to Lupus: Synchronous Estrogen Receptor alpha and RNA Polymerase II Binding within the Immunoglobulin Heavy Chain Locus. *Viral Immunol.* 2020, 33, 307–315. [CrossRef] [PubMed]

142. Sealy, R.E.; Jones, B.G.; Marion, T.N.; Vogel, P.; Hurwitz, J.L. Complex sex-biased antibody responses: Estrogen receptors bind estrogen response elements centered within immunoglobulin heavy chain gene enhancers. *Int. Immunol.* 2019, 31, 141–156. [CrossRef] [PubMed]

143. Li, K.; Li, Z.; Wohlford-Lenane, C.; Meyerholz, D.K.; Channappanavar, R.; An, D.; Perlman, S.; McCray, P.B., Jr; He, B. Single-Dose, Intranasal Immunization with Recombinant Parainfluenza Virus 5 Expressing Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Spike Protein Protects Mice from Fatal MERS-CoV Infection. *mBio* 2020, 11, e00554-20. [CrossRef]

144. Rossi, S.L.; Comer, J.E.; Wang, E.; Azar, S.R.; Lawrence, W.S.; Plante, J.A.; Ramsauer, K.; Schrauf, S.; Weaver, S.C. Immunogenicity and Efficacy of a Measles Virus-Vectored Chikungunya Vaccine in Nonhuman Primates. *J. Infect. Dis.* 2019, 220, 735–742. [CrossRef] [PubMed]