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Influenza B, C and D Viruses (*Orthomyxoviridae*)

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Glossary

EC50 Half maximal effective concentration. EC50 describes the concentration of a substance that gives half maximal response of a biological pathway.
GISRS Global Influenza Surveillance and Response Systems. GISRS is the joint collaboration of WHO with 6 WHO Collaborating Centers, four Essential Regulatory Laboratories and National Influenza Centers in more than 140 countries dedicated to protect people from the threat of influenza. It was established in 1952.
HA Hemagglutinin.
HEF Hemagglutinin-esterase-fusion protein. HEF mediates host cell attachment and fusion of the incoming virions as well as progeny virion release after completion of the replication cycle.
IBV Influenza B virus.
IC50 Inhibitory concentration 50. IC50 describes a concentration of a compound sufficient to limit virus titers by 50% when added to a host or a host cell.
ICV Influenza C virus.
IDV Influenza D virus.
NA Neuraminidase.
NEP Nuclear export protein.
NLS Nuclear localization signal. NLS describes a topogenic amino acid sequence that directs the import of a protein into the nucleus of a eukaryotic cell.
NS1 Non-structural protein.
PKR DsRNA-activated protein kinase.
RIG-I Retinoic acid induced gene I.
SC35 Serine/arginine-rich splicing factor 35.
vRNP Viral ribonucleoprotein. The genomic RNAs of influenza viruses are complexed with the viral nucleoprotein and polymerase into vRNPs that represent the smallest unit for expression of the encoded protein(s).
WHO World Health Organization.

Classification (Compact)

Influenza B virus (IBV) was identified for the first time during an outbreak of an acute respiratory disease in 1940 in Northern America, in which it was found to lack antigenic cross-reactivity to influenza A virus (IAV). IBV is classified as the only member of the genus influenza virus B within the order of the *Orthomyxoviridae*. IBV was estimated to have diverged from IAV around 4000 years ago based upon the calculated rate of amino acid substitutions within HA proteins, but the mechanisms of replication and transcription, as well as functionality of most viral proteins appear to be largely conserved, with some unique differences. IBV has been detected in rare occasions in seals and pigs, but humans are the major host species of IBV, in which it causes typically the same spectrum of symptoms of influenza-like illness as IAV although pathologies outside the respiratory tract have been determined in a minority of IBV infections. A large recent metagenomic study detected an IBV-like virus in a fish species suggesting that the genus may have additional vertebrate host reservoirs outside mammalian species. Sentinel surveillance detects IBV in some seasons as the dominating circulating influenza virus type highlighting its significant socio-economic impact. There are currently two lineages of IBV (B/Yamagata-like and B/Victoria-like viruses) with limited antigenic cross-reactivity. Viruses of both lineages circulate to varying extents and ratios in all parts of the world. The lack of a large animal host reservoir able to foster the appearance of pandemic variants, a lower evolutionary rate and generally considered milder courses of disease in comparison to IAV infection are typical features of IBV.

Influenza C virus (ICV) was first isolated in 1947 from a human patient having mild respiratory symptoms. Since the virus showed no cross-reactivity with antisera against influenza A or B viruses, it was classified as a new genus of the *Orthomyxoviridae* (influenza C virus). The virus has a worldwide distribution and the majority of humans develop antibodies against ICV early in life. Humans are the main reservoir of ICV, but occasionally the virus may also infect dogs and pigs.

In 2011 a new Influenza C-like virus was isolated from clinically ill pigs. Since this isolate is unable to reassort with ICV it is now officially named as influenza D virus (IDV). Subsequent studies showed that IDV can also infect small ruminants and cattle, the latter of which is apparently its main reservoir.

The first part of this article reviews current knowledge on type B influenza virus, whereas the second part addresses important aspects of type C and D viruses.

Influenza B virus

Virion Structure

IBV virions show pleomorphic structures that can have spherical, irregular or filamentous appearances and are surrounded by a membrane, in which the viral membrane proteins HA, NA, NB and BM2 are integrated. Type A and B influenza viruses cannot be distinguished based upon their morphology or size as determined by electron microscopy (EM) (Fig. 1). The average size of spherical IBV virions of the prototypic B/Lee/40 strain grown in embryonated chicken eggs was determined to be 137 ± 27 nm. Apparently, different organizational patterns have been reported for the eight viral negative-strand RNA gene segments, which together with the viral nucleoprotein and polymerase exist as viral RNPs inside IBV virions. Analysis of ultrathin sections by transmission electron microscopy suggested that virions of laboratory and clinical IBV strains package mostly eight RNP structures that are organized in a 7 + 1 configuration with a central long RNP encircled by 7 others. In contrast, 3D reconstructions by cryo-EM tomography were rather indicative for a disordered organization of IBV vRNPs. Studies of purified virions by electron microscopy revealed the presence of 400–500 spike-like projections per spherical virion corresponding to the viral glycoproteins. Quantitative analysis of purified virions by mass spectrometry showed that M1 is the most abundant IBV virion component followed by NP, HA and NA, whereas the level of NB and P proteins were the lowest.

Genome

The genome of IBV is organized into eight single-stranded RNA molecules that are of negative polarity and vary in length from 1.1 to 2.4 kb (Table 1). Each of the segments carries characteristic highly conserved sequences of 10 (AGUAG (A/U) AACA) and 9 nucleotides (UCGUCUUCG) at their 5′ and 3′-termini, respectively, which themselves flank short segment-specific non-coding regions (NCR) that encompass the coding sequences and short U-rich elements directing polyadenylation. Each IBV gene segment

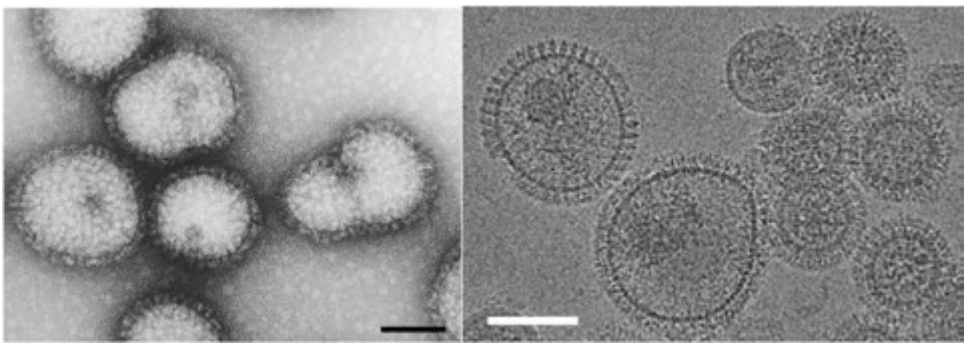

Fig. 1 *EM pictures of influenza B and C viruses*. Left: Influenza B virus. Negative staining EM. (B/Harbin/7/94, Source: RKI, ZBS4). Right: Influenza C virus. Cryo EM of C/Johannesburg/1/66. *Source*: Kai Ludwig (Core Facility BioSupraMol of the Free University Berlin). Scale bar = 100 nm.

Table 1 Identities and lengths of genome segments and encoded gene products from prototypic influenza B virus, influenza C virus and influenza D virus strains

Segment	*B/Lee/40*[a] *vRNA (nts)*	*B/Lee/40*[a] *Protein(s) (aa)*	*B/Colorado/06/2017 (Vic)*[a] *Protein(s) (aa)*	*B/Phuket/3073/2013 (Yam)*[a] *Protein(s) (aa)*	*C/Jhb/1/1966*[a] *Protein(s) (aa)*	*D/swine/Ok/1334/2011*[b] *Protein(s) aa*
1	2396	PB2 (769)	PB2 (770)	PB2 (770)	PB2 (774)	PB2 (772)
2	2368	PB1 (752)	PB1 (752)	PB1 (752)	PB1 (754)	PB1 (753)
3	2304	PA (726)	PA (726)	PA (726)	P3 (709)	P3 (710)
4	1882	HA (584)	HA (583)	HA (584)	HEF (655)	HEF (664)
5	1841	NP (560)	NP (560)	NP (560)	NP (565)	NP (552)
6	1557	NA (466)	NA (466)	NA (466)	M1 (242)	M1 (246)
		NB (100)	NB (100)	NB (100)	CM2 (115)	CM2 (?)
7	1191	M1 (248)	M1 (248)	M1 (248)	NS1 (246)	NS1 (243)
		BM2 (109)	BM2 (109)	BM2 (109)	NS2 (182)	NS2 (184)
8	1096	NS1 (281)	NS1 (282)	NS1 (281)		
		NEP (122)	NEP (123)	NEP (122)		

[a]*source*: Influenza Virus Database, NCBI (https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database).

[b]*source*: Hause, B.M., Collin, E.A., Liu, R., *et al*., 2014. Characterization of a novel influenza virus in cattle and Swine: Proposal for a new genus in the *Orthomyxoviridae* family. Mbio 5, e00031-14.

Abbreviations: nts, nucleotides; aa, aminoacids.

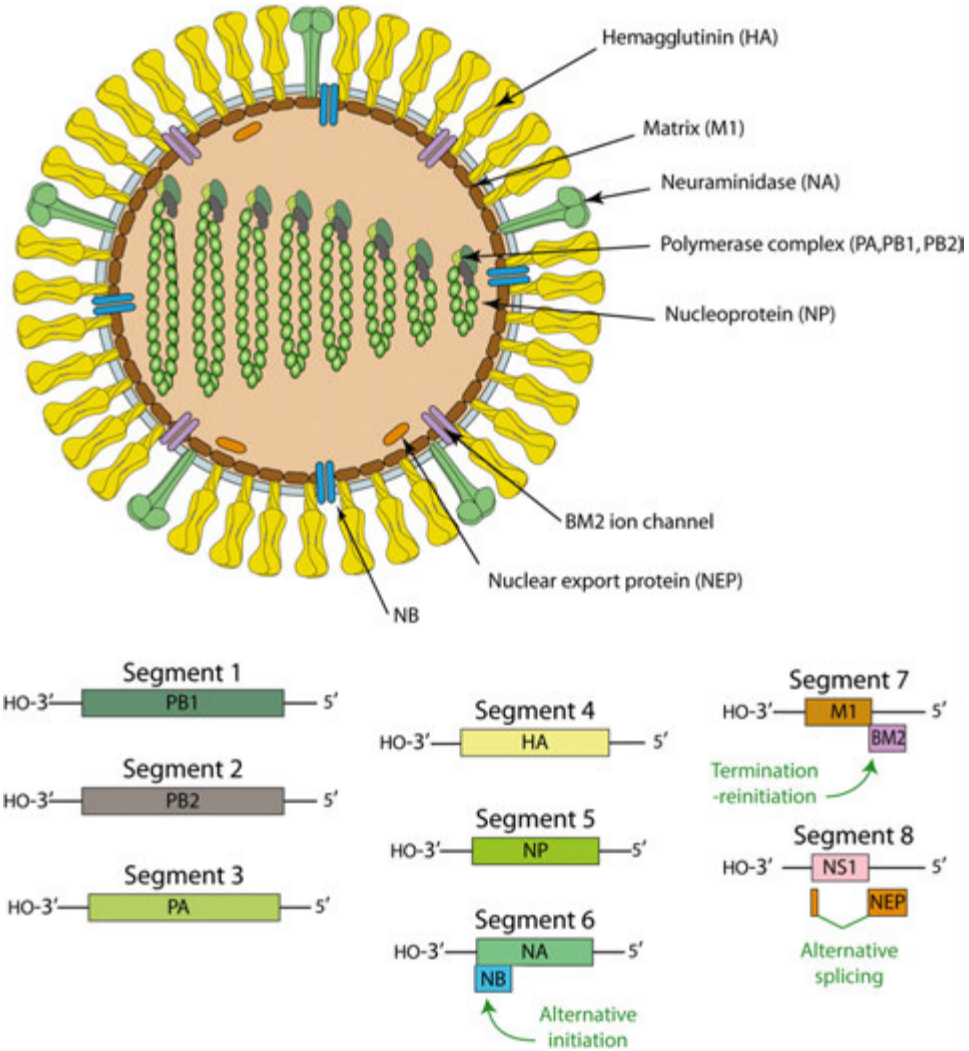

Fig. 2 *Scheme of an IBV particle and its genome segments.* Adapted from viralzone https://viralzone.expasy.org/80?outline=all_by_species with permission.

encodes one or two of the eleven known viral gene products (PB1, PB2, PA, HA, NP, NA, NB, M1, BM2, NS1, NEP) utilizing distinct strategies to expand viral coding capacity **Fig. 2**.

The nomenclature of the viral gene segments resembles those of IAV, with the largest three encoding the polymerase subunits PB2 (segment 1), PB1 (segment 2) and PA (segment 3), followed by the monocistronic HA (segment 4) and NP genes (segment 5). The residual three segments each encode for two viral proteins, which is achieved by different strategies: Segment 6 differs from its IAV counterpart by encoding not only the viral neuraminidase (NA), but also the fourth viral transmembrane glycoprotein NB in an overlapping reading frame. Its translation is initiated at the first AUG start codon, just four nucleotides upstream of the NA start codon. The NA and NB open reading frames overlap for 292 nucleotides. Translational initiation at the NA start codon is efficient despite being located downstream of the NB start.

Also the expression strategy of segment 7 encoding the viral M1 matrix and the BM2 ion channel protein differs from the one used by IAV, in which the primary mRNA transcript is processed by cellular splicing machinery proteins. In contrast, the IBV M1 and BM2 proteins are both translated from a collinear transcript in which the M1 stop codon and the BM2 start codon are assembled into a *UAA*UG pentanucleotide, which facilitates ribosomal re-initiation of protein synthesis following completion of M1 translation. Translation of BM2 coding sequence depends on the presence of a conserved *cis* element of 45 nucleotides preceding the stop-start signal. This element contains a nucleotide stretch complementary to helix 26 of 18S rRNA, which has been suggested to facilitate the recruitment of the 40S ribosomal subunit to the mRNA upon termination at the M1 stop codon.

The NS segment (segment 8) of IBV encodes NS1 and NEP proteins, which are expressed either from a collinear mRNA (NS1) or from a spliced transcript, from which an intronic sequence is removed (NEP). NEP exon 1 mRNAs utilizes the AUG start and ten codons of the NS1 reading frame, which is directly spliced to exon 2 that overlaps with the NS1 coding region for 155 nucleotides

in a +1 reading frame. No homologs of accessory protein genes identified in IAV such as PB1-F2 or PA-X have been reported for IBV.

Reassortment, i.e., the exchange of gene segments between two viruses infecting one cell, is a general evolutionary mechanism of segmented RNA viruses, which is also a major driving force for the appearance of novel epidemic IBV strains both from within and in between the Victoria- and Yamagata lineages (see below). Classical coinfection experiments indicated that there can be phenotypic mixing of type A and B viruses generating particles that contain antigenic determinants derived from both parental types. However, IBV has never been observed to produce stable reassortant viruses harboring a gene segment(s) of IAV and vice versa. At least some part of this restriction appears to operate on the level of genome incorporation. Recombinant viral gene segments carrying the IBV HA or NA reading frames could be incorporated together with six other IAV segments into infectious chimeric influenza A/B viruses, when the coding regions were flanked with the packaging signals of the type HA and NA segments. The unique packaging signals of each of the viral gene segments have been mapped for IAV to contain the non-coding regions (NCRs) as well as terminal parts of the coding sequences, but have been less well studied for IBV. The appearance of defective interfering (DI) RNAs derived from viral gene segments containing the conserved terminal promoter sites with large internal deletions of the coding regions, especially in cells infected at high multiplicity, has also been described for IBV. However, the precise mechanism(s) by which the viral polymerase generates viral DI RNA remain to be elucidated.

Life Cycle and Functions of Viral Proteins

Virus Entry and Genome Expression and Replication by Polymerase Proteins

The main targets of IBV are the epithelial cells lining the upper respiratory tract. The available evidence indicates that the replication cycles of IBV and IAV follow very similar or even identical principles, which are, however, executed by divergent and a few type-specific proteins, respectively, resulting in some virus type-specific mechanisms that are described in detail below. Hence, IBV and IAV were shown to enter a permissive host cell by receptor-mediated endocytosis, which is initiated by binding of the viral hemagglutinin (HA) to sialic acid residues that are present either as glycoprotein or glycolipids on the host cell surface. Intracellular release of the viral genome requires both acidification of the interior of the virion through the viral BM2 ion channel, which is followed by HA–mediated fusion of the viral and endosomal membranes in the acidic environment of the late endosome. The pH optimum for fusion activity of the IBV HA has been determined at pH of 5.4–5.6, which is slightly above the range known for HA of human IAV (pH = 5.0–5.4). The viral RNPs are transported from the cytosol into the nucleus, where the viral RNA polymerase, a trimeric enzyme of ca. 270 kDa initiates transcription and replication of the viral gene segments. These events have been less well studied in cells infected with IBV compared to type A viruses. However, the high conservation of structural elements within the three polymerase subunits PB1 (enzymatic core), PB2 (cap binding domain, aa 318–480) and PA (endonuclease site, aa 1–195) as determined by X ray crystallography, as well as functional studies of recombinant polymerases strongly argue for a conserved mode of action.

Within the nucleus each vRNP acts as an independent unit that can either be transcribed into polyadenylated positive-sense viral mRNA using a cap-snatching mechanism, or serves as the template for synthesis of a positive-sense complementary RNA (cRNA) which in turn directs the replication of another virion RNA (vRNA) molecule. The IBV PB2 subunit binds not only to methylated G-capped RNAs like its IAV counterpart, but also to unmethylated GpppG caps indicating a larger flexibility of the IBV PB2 cap binding domain. The viral RNA polymerase complex binds the vRNA or the cRNA at the promoter sites located on the highly conserved stretches of nearly complementary nucleotides at the 3′- and 5′-termini. Successful expression and purification of soluble recombinant IBV polymerase has recently facilitated detailed in vitro studies of binding parameters to viral vRNA and cRNA mimics as well as characterizations of the enzymatic activities of IBV P proteins. It is generally believed that influenza viruses express an error-prone RNA polymerase which contributes to high viral evolutionary rates, but this has not been determined experimentally for the IBV polymerase.

The major component of the vRNPs is the viral nucleoprotein (NP) that is a basic (pI > 9.0) 62 kDa protein and wraps around the viral RNA. It is an essential cofactor for viral transcription and replication and it consists of a head domain, a body domain and a tail loop like its IAV counterpart, to which it has sequence identity below 40%. The tail loop of one NP monomer can insert into a neighboring monomer, which drives the formation of homo-oligomers. A distinct feature of the IBV NP protein is an extended N-terminal region of 70 amino acids that appeared intrinsically disordered in structural analyzes. In a study by NMR and small-angle X ray scattering the N-terminal NP region was bound to nuclear import factor importin-α7, which together with biochemical and mutational analyzes suggested that the N-terminal region of NP that is enriched in basic amino acids between positions 30 and 71 directs its nuclear targeting.

Intracellular expression of viral mRNA directs the synthesis of eleven known IBV proteins that facilitate amplification and nucleo-cytoplasmic export of the viral genome, the production of progeny virions and/or manipulate innate antiviral defenses. The newly synthesized vRNPs are exported to the cytosol with the support of the viral NEP and, possibly, the M1 protein, which both contain nuclear export signals. The nuclear export of vRNPs is sensitive to inhibition by leptomycin B indicating a dependency on the exportin 1-dependent pathway. IBV encoded NS1 protein suppresses the stimulation of cytosolic pattern recognition receptors such as RIG-I and PKR through newly replicated vRNPs. IBV replication triggers an apoptotic response in infected cells and the activation of both extrinsic and intrinsic induction pathways has been described. The assembly and budding of IBV progeny

virions occurs at the plasma membrane, which is followed by virion release that depends on NA activity. In the following we will discuss the remaining structural and regulatory IBV gene products with a focus on their virus type-characteristics.

Hemagglutinin (HA)

The HA of IBV is synthesized in the ER as a HA_0 precursor of approx. 63 kDa with a predicted leader peptide of 15 amino acids that needs to be proteolytically cleaved by the cellular signal peptidase. HA_0 precursor is cleaved by trypsin-like proteases into HA_1 (approx. 346 amino acids) and HA_2 subunits (approx. 223 amino acids) to become biologically active. HA has an essential role in the viral life cycle as it mediates binding of the virion to host cell surface receptors as well as promoting the release of the viral genome from the late endosome. The HA of IBV is heavily glycosylated containing 10–12 N-linked glycosylation sites and it is assembled into homotrimers. The high number of glycan attachment sites may reflect the prolonged circulation of IBV in humans as glycosylation is believed to be a strategy, by which influenza viruses mask antigenic sites. Cleavage activation of the HA occurs within a motif that is highly conserved among viruses of the Yamagata and Victoria lineages (PAKLLKER↓GFFGAIAGFLE), which liberates the hydrophobic fusion peptide at the N-terminal end of HA_2. Trypsin and the serine proteases TMPRSS2 and TMPRSS11d were shown to execute HA cleavage activation in vitro. TMPRSS2 knock out mice were shown susceptible to IBV infection suggesting the existence of additional proteases that can activate the HA. Several X ray structures have been reported for the neutral pH form of HA expressed by the divergent IBV lineages (**Fig. 3**). Despite a low degree of sequence identity of approx. 25% to IAV HA these studies revealed an overall conserved fold of the trimeric HA ectodomain composed of a membrane-distal globular head domain (mainly located on HA_1) and an elongated stem (mainly located on HA_2) formed by each monomer. Each head domain carries a receptor binding pocket that is built by the 190 helix (aa 193–202) on the top, the 240 loop (aa 237–242) as the left boundary and the 140 loop (aa 136–143) as the right edge with additional contributions by the conserved Phe 95, Trp 158 and Tyr 202 residues. The principal receptor determinants of IBV HA are sialic acid (SA) conjugated to cellular surfaces. An interesting variation among clinical IBV isolates has been noted in terms of their receptor specificity by glycan array analysis. 19 of 21 Yamagata-like viruses isolated between 2001 and 2006 in Taiwan preferentially bound to α-2,6 linked sialic acid that is also the

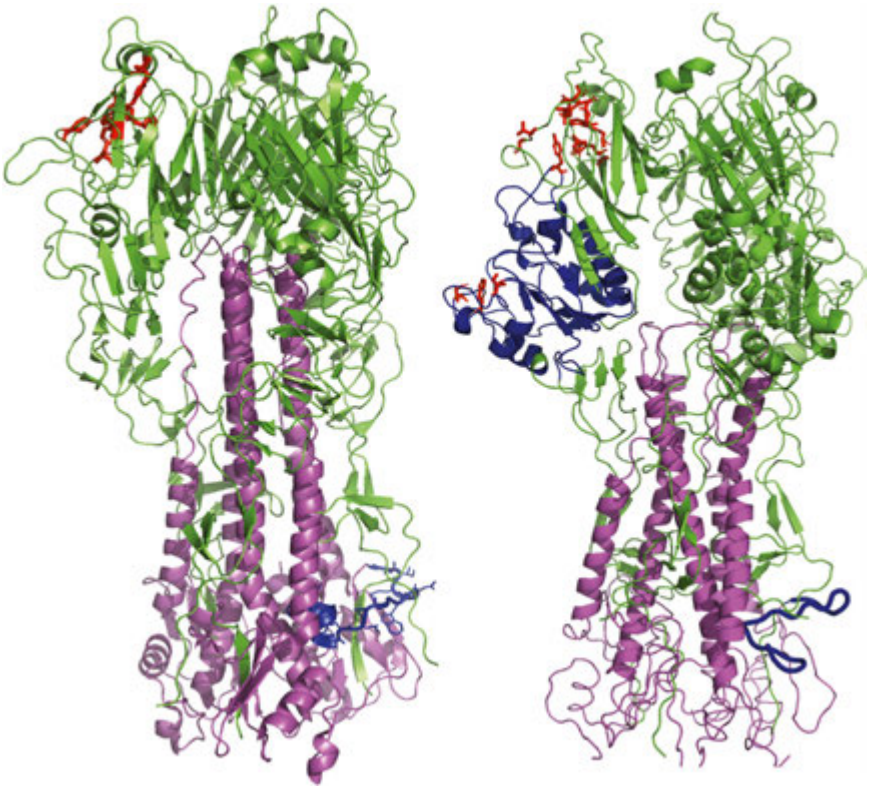

Fig. 3 *Crystal structure of an HA (left) and HEF (right) trimers.* Polypeptide chains are displayed as green (HA_1, HEF_1) and magenta cartoons (HA_2, HEF_2). HA (left): The amino acids contributing to receptor-binding (Phe-95, Ser-140, Trp-158, His-191, Tyr-202, Pro-238 and Ser-240) are shown as red stick in one HA_1 subunit. The fusion peptide at the N-terminus of the HA_2 subunit is displayed as blue sticks. HEF (right): The esterase domains shown in blue within one HEF_1 subunit. The catalytic triad of the esterase domain (serine 57, aspartate 352, histidine 355) is shown as red sticks. The amino acids contributing to receptor-binding (Tyr 127, Thr 170, Gly 172, Leu 184, Thr 186, Phe 225, Tyr 227, Arg 236, Phe 293) are shown as red stick in one HEF_1 subunit. The fusion peptide at the N-terminus of the HEF_2 subunit is displayed as a blue line. Figures were created with Pymol2.2 (https://pymol.org/2/) from pdb file 4M40 showing HA from influenza virus B/Yamanashi/166/1998 and from pdb file 1FLC containing C/Johannesburg/1/66.

major receptor determinant for IAV in the human upper respiratory tract. In contrast, 12 of 23 Victoria-like viruses additionally bound α-2,3 linked sialic acid, which is the receptor for avian IAV and can be found in humans mainly in the lower respiratory tract (LRT). 6 of 23 Victoria-like IBV recognized specifically sulfated glycans. Receptor specificity is considered an important determinant of influenza virus pathogenesis, but it remains to be established whether IBV strains with dual receptor specificity are more prone to infect the LRT which is usually associated with more severe symptoms.

The HA is a main target for antibodies elicited after infection or immunization. Phylogenetic and structural analyzes revealed four major canonical antigenic sites reflecting the strong immune pressures on the IBV HA. Antigenic sites have been mapped to the 120 loop (116 – 137), the 150 loop (141 – 150), the 160 loop (162 – 167), and the 190 helix (194 – 202) which contain sequence-variable immunodominant epitopes. The IBV HA protein contains a highly conserved cytosolic tail domain of 10 amino acids (RDNVSCSICL) with important regulatory roles in virion assembly and budding. The two cysteine residues are modified by palmitoylation thereby increasing hydrophobicity of the tail. Removal or shifting of palmitoylation sites affected membrane fusion activity of the HA. Studies by reverse genetic analyzes suggested that the cytosolic tail is important for the incorporation of IBV HA into virions and localization of viruses in lipid raft microdomains during entry. A deletion in the HA tail did not affect the fusion activity of HA, but yet it strongly reduced viral propagation and incorporation of HAs into virions. Interestingly, the phenotype of the HA truncation mutant was partially rescued by a compensatory E136K mutation in M1 suggesting that the assembly of HA into budding virions depends on an interaction with M1.

Neuraminidase (NA)

The neuraminidase, the receptor destroying enzyme of influenza A and B viruses, is a type II transmembrane glycoprotein found on the surface of virus-infected cells and in the membrane of the virions. Its main function is thought to be the removal of inhibitory receptor determinants from mucus in the respiratory tract to facilitate virion docking to the host cell, as well as to mediate the release of progeny virions from the host cell, which is the last step of the virus replication cycle. Hence, inhibition of NA by small molecules or antibodies diminishes virus propagation. There are many structural similarities between the NA molecules of type A and B viruses. In both viruses NA matures into a tetrameric structure despite the low degree of sequence identity of 30%. The monomers form a head domain composed of six-bladed β-propellers that are linked to membrane-proximal elongated stalks, which together build a box-like tetramer. The enzymatic and antigenic sites are located in the head domain. The eight NA amino acids that directly bind to sialic acid and are important for the catalytic activity of NA (R118, D151, R152, R224, E276, R292, R371, and Y406; N2 numbering) are highly conserved among type A and B viruses. NA is the main target for current antiviral therapy of human infections by circulating influenza A and B viruses (see below).

NB

NB is an enigmatic second transmembrane glycoprotein encoded by segment 6 with an unknown function. It consists of an N-terminal ectodomain of 18 amino acid, a predicted 22 amino acid transmembrane domain and a cytosolic part of 60 amino acids. It has two glycosylation sites in the ectodomain. Mature NB forms a tetrameric structure and is incorporated into virions, but only in low amounts as determined by quantitative mass spectrometry. NB transport to the cell surface was suggested to depend on palmitoylation of cysteine 49. Most interestingly, a recombinant influenza B/Florida/04/2006 virus engineered to lack NB expression by introduction of an early stop codon not affecting NA expression replicated well in stable cell lines or primary tissue cultures. The NB-deficient mutant virus had no specific phenotype in mice and propagated in a ferret model in virtually identical manner to the wildtype virus with a retained (low) capacity for respiratory droplet transmission. These findings partially contrast with a weakly delayed viral growth for a B/Lee/40 mutant virus in which NB palmitoylation at cysteine 49 was abrogated, which may have affected trafficking of NB to the cell surface. Yet, the likely essential function of the NB protein in IBV replication, pathogenesis or transmission, which is expected by the strong conservation of the NB reading frame, remains to be discovered.

Matrix protein (M1)

The M1 proteins are the most abundant morphogenic virion proteins of IBV and IAV. The IBV M1 protein has a conserved length of 248 amino acid and it shares about 30% sequence identity with its type A virus counterpart. The physicochemical properties, domain structure and regulatory roles of the IAV M1 protein in facilitating nucleocytoplasmic export of vRNPs late in infection have been well studied, but considerably less is known about the characteristics of the IBV M1. However, IBV M1 has been suggested to engage into a binding interaction with the viral BM2 protein and that this interaction is important for incorporation of M1 and vRNPs into virions. Mutational analysis identified within the IBV M1 protein a bipartite nuclear localization signal (amino acids 74–94) and two leucine-rich exportin 1-dependent nuclear export signals at positions 3–14 and 124–133. However, the role of these topogenic sequences in the viral replication cycle remain to be determined. In case of IAV the M1 protein is thought to bridge an interaction between vRNP and the viral nuclear export protein (NEP), which facilitates nuclear export of the viral genome via the exportin 1-pathway. However, the IBV NEP has a reported ability to bind directly to vRNPs suggesting that M1-mediated complex formation would be dispensable or redundant for IBV.

BM2

BM2 is a type III transmembrane protein that provides a proton channel function essential for the intracellular release of vRNPs from late endosomes. BM2 consists of a short 7 amino acid ectodomain, a 19 amino acid transmembrane domain and a cytosolic domain of 83 amino acids, and it exists in the form of a homotetramer. BM2 is a functional homolog with the IAV M2 protein to

which it has little sequence identity apart from a conserved 19-HxxxW-23 proton channel motif. BM2 was recently shown to efficiently complement the multi-cyclic growth of a recombinant M2-deficient influenza A virus, which was otherwise confined to a single cycle infection. BM2 differs from the M2 protein in being insensitive to channel blockers such as amantadine resulting in natural resistance of type B influenza viruses to this first generation class of anti-influenza compounds. The resistance to amantadine was explained by differences in the interior part of the proton channel, which is covered by polar amino acids and not hydrophobic residues as in the case of the IAV M2 protein, as well as by a reduced size of the channel pore. Apart from serving as a proton channel BM2 protein has also important morphogenic activity since recombinant viruses with C-terminal BM2 deletions of as little as six amino acids displayed irregularly formed virions and the virus was strongly attenuated in its replication ability. The large cytosolic BM2 domain adopts a coiled-coil tetramer. Biochemical analyzes showed that the C-terminus of BM2 is important for efficient membrane association of the M1 protein in flotation centrifugation, as well as incorporation of M1 and vRNPs into virion particles. Within the cytosolic BM2 domain, there is an electrostatic interaction with a positive charged N-terminal part (residues 44–71) and a negative C-terminus (72 – 103), the latter of which has been suggested to bind to the IBV M1 protein. From these analyzes, a model emerges in which the BM2 protein is a central factor that recruits viral RNPs and the M1 protein to virion assembly sites as a prerequisite for efficient budding. Whether BM2 also contributes to scission of virus particles from the plasma membrane as described for M2 for IAV remains to be investigated.

Non-structural protein 1 (NS1)

The NS1 protein is abundantly expressed in virus-infected cells and the main antagonistic protein enabling IBV to interfere with the innate type I interferon (IFN) defense and to propagate efficiently in mammalian hosts. Hence, there are strong parallels to the functions of the IAV NS1 protein although they share a sequence identity of less than 25%, and there are also type-specific activities of the two NS1 proteins. The IBV NS1 protein has a conserved length of 281 amino acids with a predicted molecular mass of 32 kDa. It is described as a non-structural protein although low levels of NS1 were detected in purified virions by mass spectrometric analysis. Early biochemical analyzes identified in the N-terminal NS1 domain an RNA binding activity (amino acids 1–93) and the ability to bind to the IFN-induced protein ISG15 (1 – 103), a ubiquitin-like modifier of nascent proteins. Later on, it was found that the C-terminal part of NS1 (94 – 281) can also bind RNA. Reverse genetic analysis confirmed that in epithelial cells the NS1 protein inhibits the accumulation of IFN-β transcripts and activation of IRF3, a central transcriptional regulator of IFN-β expression. For this activity the C-terminal NS1 domain was essential. However, IBV is a stronger activator of type I and III IFN expression early in infection compared to IAV. This depends on the cellular pattern recognition receptor RIG-I and it is possibly caused by a more rapid release of vRNPs from endosomes. Interestingly, the IBV NS1 protein does not bind RIG-I, but rather targets the ubiquitin E3 ligase TRIM25 that activates RIG-I by ubiquitination, whereas the type A NS1 protein binds RIG-I and TRIM25 directly. Phenotypic analyzes of recombinant mutant IBV demonstrated that NS1 facilitates efficient viral propagation in vitro and in vivo by blocking the activation of the central immune kinase PKR that can shut-off protein synthesis in infected cells. Silencing of PKR strongly depended on a binding interaction of the N-terminal NS1 domain with the kinase domain of PKR. Structural information from X-ray crystallography has been reported for the N-terminal domain (15 – 93) of NS1 that adopts a six helical dimeric fold and a large C-terminal fragment (141 – 281). It is uncertain whether the IBV NS1 protein forms higher order structures as was recently shown for the type A NS1. ISG15 has been shown to regulate influenza A and B virus infections. It was therefore not surprising to find out that the IBV NS1 protein not only binds ISG15, but it can also prevent its conjugation to target proteins. Interestingly, the IBV NS1 protein binds strongly to the human ISG15 and only poorly to ISG15 homologs in other mammalian species, which provides an important example of a species-specific viral antagonism of the type I IFN response.

The intracellular localization of the IBV NS1 protein is highly dynamic throughout the infection. At early times of infection, NS1 accumulates in nuclear SC35-positive speckle domains enriched in RNA processing factors and leads to a rounding of their usually irregular appearance. This activity was localized within the N-terminal 90 amino acids which also displays a monopartite NLS at position 46–57 inside the N-terminal RNA binding domain. However, speckle association of NS1 is resolved in late phases of infection when vRNPs are exported to the cytosol, and is accompanied by a cytosolic relocalization to downregulate cellular immune receptors such as PKR and RIG-I. It has been speculated that the transient interaction of the IBV NS1 protein with speckles reflects a role in promoting export of viral mRNAs. The IBV NS1 protein lacks two important properties, which are found in IAV NS1 proteins: First, the absence of interference with the cellular polyadenylation machinery, which is mediated by binding to a 30 kDa subunit of cleavage and polyadenylation factor. Second, IBV NS1 has the inability to activate phosphatidylinositol-3-kinase (PI3K) signaling by binding to its p85beta subunit, which has been suggested to prevent premature apoptosis in infected cells.

NEP

The IBV NEP protein has a conserved length of 122–123 amino acids. In spite of its similar size the sequence identity of IBV NEP with IAV NEP is below 25%. However, both NEPs have conserved functions as vRNP export factors and regulators of the viral RNA polymerase. The IBV NEP has been shown to interact with human nucleoporins and exportin 1, and this interaction has been mapped in the hydrophobic N-terminal region at positions 10–19 of NES. In contrast to IAV, the IBV NEP has been reported to bind directly to vRNPs, and may, hence, be able to facilitate nucleocytoplasmic export of the viral genome independently of the M1 protein. An additional activity of NEP in regulating polymerase protein functions was observed in minigenome reporter assays, in which increasing amounts of NEP reduced the accumulation of viral mRNA and enhanced cRNA levels without affecting vRNA levels. Neither the precise target of IBV NEP within the viral RNPs nor the mechanism underlying this regulation have been established.

Epidemiology

IBV is considered to cause seasonal influenza epidemics every 2–4 years, but less comprehensive systematic analyzes on its epidemiology are available compared to IAV. Two IBV lineages derived from the prototypic B/Victoria/2/87- and B/Yamagata/16/88-strains co-circulate with the IAV H1N1 and H3N2 subtypes to cause seasonal influenza. However, the extent of human infections for each of the four viruses is highly variable and can differ from one geographic region to the other even in the same epidemic season. Sentinel surveillance data in Europe since 2001 showed that the detection of IBV among circulating influenza viruses ranged from 1% to 70% through all age groups and that IBV was in five seasons the most prevalent virus type. A long-term global comparison suggested that IBV is the most prevalent influenza virus type affecting older children (5–17 years) and is second behind A(H3N2) in the elderly (> 65 years). Moreover, in Germany the rates for hospitalization attributable to IBV infection were estimated to be 81 per 100.000, which was in between the rates for A(H3N2) and A(H1N1) influenza (99 and 56 per 100.000). Significantly, IBV infections were the main cause of mortality attributable to influenza in the US in four epidemic seasons between 1997 and 2009. IBV was detected in 1222 (73%) of the 1674 fatal influenza cases reported through the German Public Health notification system for infectious diseases in the severe 2017/2018 season. Taken together, studies show that IBV imposes a substantial burden of disease.

The IBV HA genes evolve at a rate of 2.0×10^{-3} substitutions/site/year, which is somewhat slower compared to the seasonal IAV HA subtypes H3N2 (5.5×10^{-3} substitutions/site/year) and H1N1 (4.0×10^{-3} substitutions/site/year). This finding correlates with a slower antigenic drift of IBV and may indicate higher structural and/or functional constraints on its HA. The B-Yamagata and B-Victoria lineages split in the 1970s and the lineages were distinguished based upon genetic and antigenic differences of their HA and NA genes (Fig. 4). During their co-circulation multiple reassortment events occurred either within or in between the lineages. Recent molecular genetic analyzes indicated that only the HA, PB1 and PB2 genes continue to evolve in a lineage-specific manner. Current viruses with the Yamagata-like HA have diverged into clades 2 and 3, whereas most strains with a

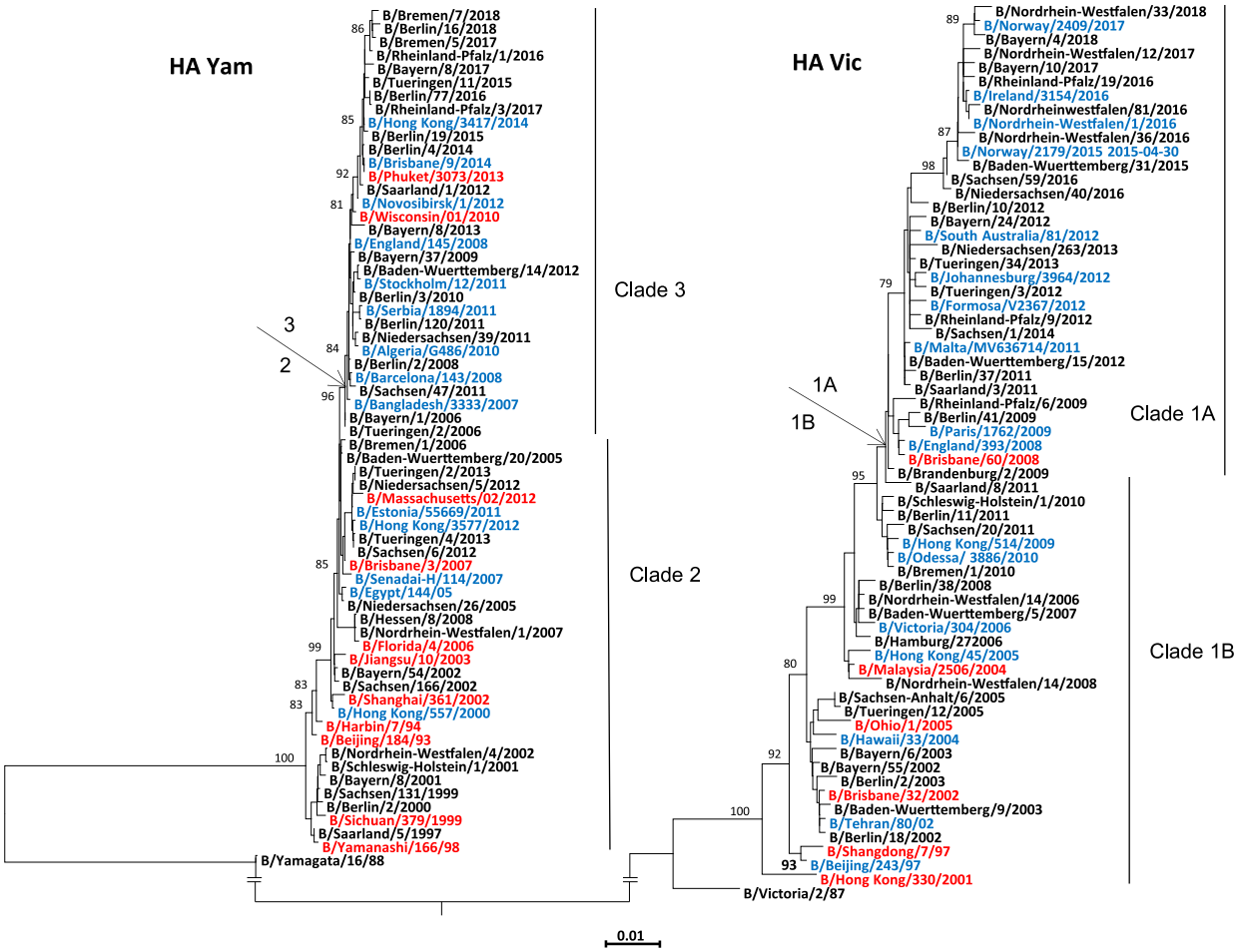

Fig. 4 *Phylogenetic tree of IBV HA genes with representatives of B-Yam and B-Vic lineage using the Neighbor-joining method*: Vaccine viruses (red), reference viruses (blue) and circulating isolates (black) are shown. The continued evolution of IBV HA genes is presented on https://nextstrain.org.

Victoria-like HA belong to clade 1A. There is low antigenic cross-reactivity between the two IBV lineages and often circulating strains and the IBV vaccine component in trivalent vaccines have not matched well.

Clinical Features

Infections by type B or A viruses cause febrile influenza-like illness characterized by a sudden onset of fever accompanied by cough, nasal congestion, headache, myalgia and other types of malaise that appear after a typical incubation period of 1–3 days. There is a large spectrum in the severity of symptoms ranging from an inapparent infection with no virus shedding to a fulminant disease, which may depend on the immune status, underlying risk factors, and age of the patient. Some studies on pediatric populations reported slightly increased frequencies of myalgia, sore throat and hoarseness in IBV infection compared to IAV, but other studies have not confirmed this observation. From outpatient-attended influenza infections or experimental analyzes there are no data suggesting that the clinical features of IBV infection are different from the ones caused by IAV. Complications of IBV infection affect the lungs (e.g., bronchiolitis or pneumonia), but have also been observed in other organ systems leading to neurological (encephalitis), muscular (myositis) or cardiologic presentations (myocarditis).

Pathogenesis

Clinical symptoms of seasonal influenza are in general similar for IBV and IAV and no unique pathogenetic mechanism has been ascribed for IBV, when it infects and spreads through the human respiratory tract. One autopsy study of 45 fatal influenza B cases showed that both lineage viruses caused inflammation of the trachea and bronchi with necrotic cells, pulmonary edema and hemorrhages as common findings. Viral antigen was mainly found in the columnar epithelium of the trachea and bronchi as well as in submucosal glands even in the absence of bacterial coinfection. These findings were similar to the ones seen in fatal influenza A virus infection. Yet, it is unclear how representative these findings from post mortem tissue are to the pathogenesis in uncomplicated influenza for which there is only little data available. The pathogenesis of IBV infection in experimental animal models depend on virus adaptation (e.g., mice), the use of specific virus strains (ferrets, guinea pigs) or in some models there is no pathological changes (e.g., Syrian golden hamsters).

Diagnosis

It is not possible to diagnose an influenza B virus infection based on clinical presentation alone as there is a large range in symptom severity and a number of other respiratory pathogens that cause similar or identical illness. Hence, laboratory testing of respiratory patient samples such as nasopharyngeal swabs or broncho-alveolar lavage fluid is required to confirm a suspected IBV infection by the presence of viral RNA or antigen. In principle, reverse transcription-dependent real-time PCR assays, viral culture or rapid point-of-care tests detecting viral antigen or the viral genome are used, which differ in sensitivity and testing time. RT-PCR assays have a high sensitivity close to 100% and can be completed within a few hours, but usually RT-PCR analyzes require the samples to be transported to a diagnostic laboratory operating the required equipment (e.g., thermocyclers). PCR primers and probes often target the M gene to detect IBV and utilize the HA sequence for discrimination between the B-Yamagata and Victoria lineages. Results from rapid antigen detection or isothermal nucleic acid amplification may be available within 30 min, but these assays have on an average slightly lower sensitivity and specificity.

Treatment

There are two classes of compounds licensed for the treatment of acute influenza A and B virus infections, which target either the viral NA or the PA subunit of the viral RNA polymerase. The neuraminidase inhibitors (NAI) include the oral oseltamivir (given as the prodrug oseltamivir phosphate), the inhaled zanamivir and the intravenous peramivir, which all target the substrate binding pocket of NA and reduce enzymatic activity in the nanomolar range to stall the spread of newly synthesized virions to other host cells. It is a consistent finding that IC50 values for IBV NA are up to tenfold higher compared to concentrations needed to inhibit IAV NA. For oseltamivir this observation has been explained by a lower flexibility of the active site residue E276 that impedes high affinity binding to the hydrophobic pocket. The development of resistance towards NAI in IBV has been observed in experimental studies as well as in patients undergoing antiviral treatment. However, the genetic barrier to acquire resistance mutations appears to be high, possibly due to a loss of viral fitness. Global surveillance by the GISRS detected NAI resistance in circulating IBV in five consecutive seasons between 2012–13 and 2016–17 at frequencies between 0.2% and 2.0% using phenotypic assays suggesting that these compounds remain suitable for clinical use. Single amino acid mutations conferring NAI-resistance in clinical isolates have been identified either directly at the catalytic site (R152K), at the NA framework (D198N) or near the sialidase center (G109E, G402S).

Baloxavir marboxil, the prodrug of an oral anti-influenza compound, was licensed for the treatment of seasonal influenza in Japan and the US in 2018. It targets the cap-dependent endonuclease activity located on the PA polymerase subunits of IAV and IBV, and the drug can be given as a single dose. The EC50 of Baloxavir acid for the propagation of IBV in MDCK cells was reported in the range of 5.6–8.5 n mol. Analyzes of A(H1N1) and A(H3N2) viruses isolated from patients in the treatment studies showed that single mutations of the conserved residue I38 to T, F or M reduced the sensitivity to Baloxavir by more than 10-fold. No resistant IBV were reported in the clinical studies, but reverse genetics showed that PA I38 mutations increase the EC50 of IBV by 2- to 7-fold. Structural analyzes showed that resistance mutations reduced the affinity of the inhibitor to PA. Strong conservation of the PA I38 position in current human influenza B viruses suggests only a low background level of resistance to Baloxavir. Importantly, no cross-resistance has been found for Baloxavir and NAI.

Prevention

Vaccination is the most effective measure to protect against seasonal influenza A and B, for which either inactivated or live-attenuated vaccines are available. Since IAV and IBV strains circulate in unpredictable patterns, killed vaccine formulations contain a mixture of inactivated whole viruses or purified HA and NA proteins from one A(H1N1), one A(H3N2) and one (trivalent influenza vaccine, TIV) or two IBV strains (quadrivalent influenza vaccine, QIV) designed to elicit a protective humoral immune response. The specific strains to be included into the vaccine of the forthcoming season are recommended by WHO twice a year based upon extensive genetic analysis and antigenic profiling of circulating virus strains. Live-attenuated vaccines contain a mixture of three or four attenuated virus strains with the same antigenic profiles. High vaccine effectiveness depends on a good antigenic match between the vaccine strains and the circulating epidemic viruses. The elderly may also have weaker immune responses against the vaccine. Since there is only limited antigenic cross-reactivity between the two IBV lineages that co-circulate since 2001 with unpredictable frequencies, it has turned out a formidable challenge to predict the specific IBV lineage component for TIV. In fact, TIV was in five of ten seasons from 2001 to 2010 reported to confer little or no protection against the circulating IBV strains in the US. Still, recent meta-regression analysis quantified that there was reduced but substantial IBV vaccine effectiveness against laboratory confirmed infection even in seasons with a type B lineage mismatch (67% for matched seasons, 35% for mismatched seasons). The suboptimal performance of TIV led to a first recommendation in 2012/2013 for the inclusion of both IBV lineages into a seasonal QIV, which have been increasingly used by different countries. Modeling studies suggested a good potential that the simultaneous coverage of both IBV lineages by quadrivalent formulations can substantially reduce the burden of influenza B infection if vaccine uptake is sufficiently high.

Influenza C and D viruses

Virion Structure and Genome

Influenza C (ICV) and D virus (IDV) particles exhibit various morphologies: elliptical, spherical with a diameter of 80–120 nm or filamentous with a similar diameter but with lengths in the μm range (**Fig. 1**). A peculiar feature of ICV and IDV particles not observed for IAV virions is the arrangement of its unique spike, the hemagglutinin-esterase-fusion glycoprotein (HEF) in a reticular structure that consist mainly of hexagons. The regular polymeric structure can also be observed in isolated HEF molecules indicating that the hexagonal arrangement is an intrinsic feature of the protein likely involving lateral interaction between its ectodomains.

The interior of the virus particle contains the viral ribonucleoprotein (RNP) complexes, which consists of negative-sense, single-stranded RNA bound to the nucleoprotein NP and to three polymerase proteins (**Fig. 5**). In contrast to IAV and IBV, the genomes of ICV and IDV consist of only seven (not eight) different segments (**Table 1**), but most virus particles nevertheless package eight RNPs arranged in the typical 1 plus 7 pattern in which a central RNP is surrounded by seven circularly arranged RNPs. It is still not known how specific packaging of genome segments is achieved, but the segmented genome provides an evolutionary advantage of allowing the exchange of individual genome segments with those of other strains.

All RNA segments are flanked by non-coding sequences, which are more variable in length than those of influenza A and B viruses. The first twelve nucleotides at each 3′ end as well as the first eleven nucleotides at each 5′ end are conserved between genome segments and are partially complementary to each other, which enables the single stranded RNAs to form "panhandle" structures. This unique structure serves as the promotor for transcription of mRNAs and plays a pivotal role in genome replication and packaging. A uridine-rich region at the 5′ end of each segment serves as the poly A tail template for mRNA transcription.

The longest three gene segments encode the proteins PB2, PB1 and PA/P3 that form the trimeric polymerase complex, which is bound to one end of each RNP. PB1 houses the polymerase active site, whereas PB2 and PA/P3 contain cap-binding and endonuclease activities, respectively, required for transcription initiation by cap-snatching, a process during which a short nucleotide sequence is cleaved from the 5′ end of cellular pre-mRNAs. The 3D-structure of polymerase proteins and the active site of the polymerase and endonuclease as determined by X-ray crystallography are conserved between influenza viruses suggesting that the mechanism of transcription and replication is very similar in all influenza virus types. The fourth segment of ICV encodes the glycoprotein HEF, the only spike of the viral membrane, which is discussed in more detail below. The fifth segment encodes

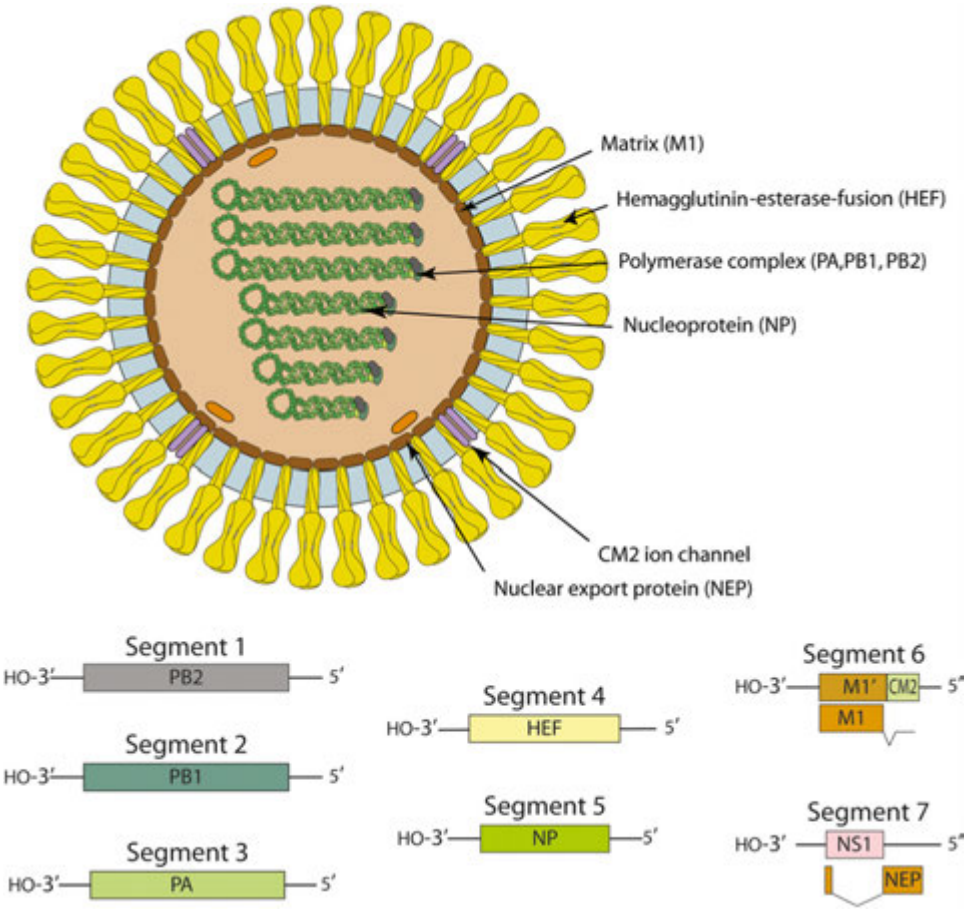

Fig. 5 *Scheme of an ICV particle and its genome segments.* Adapted from viralzone https://viralzone.expasy.org/81?outline=all_by_species with permission.

the nucleoprotein NP that associates with the viral genome segments in a double-helical conformation in which two NP strands of opposite polarity are associated with each other. The sixth segment encodes two proteins, the matrix protein M1, a peripheral membrane protein that covers the viral envelope on its inside, and CM2, a short transmembrane protein with proton-channel activity required for virus entry. M1 contributes to the morphology of virus particles and also to another unique feature of ICV, the formation of cord-like structures, very long (up to 500 μm) tubules containing HEF and M1, but no RNPs emanating from the surface of virus-infected cells.

In contrast to IAV, the larger M1 protein of ICV and IDV is translated from a spliced RNA, but the mechanisms of splicing differ between ICV and IDV. In ICV the removal of an intron generates a stop codon whereas in IDV splicing generates a second exon encoding a 4-residue peptide that is added to the primary M1 transcript. The unspliced mRNA encoding CM2 translates into a long precursor protein called M1′ or p42. P42 contains an internal signal peptide, which targets the protein into the ER. Residues C-terminal to the signal peptide are translocated into the lumen of the ER until translocation is stopped by a second hydrophobic region, the transmembrane region of CM2. The signal peptide is then cleaved by signal peptidase yielding the mature CM2 and the p31 protein, which is rapidly degraded.

The seventh vRNA encodes the two non-structural proteins NS1 (246 amino acids) and NS2 (182 amino acids) that are generated via mRNA splicing: the unspliced mRNA is translated into NS1 and the spliced mRNA translates the shorter NS2 protein. The N-terminal residues of NS1 and NS2 are identical in sequence, splicing then generates a shift in the ORF in such a way that the remaining residues are translated from a different reading frame. Similar to IAV and IBV, it is believed that NS1 counteracts the cellular interferon response and NS2, which is also designated nuclear export protein (NEP), mediates the nuclear export of RNPs. Small amounts of NEP may be incorporated into virus particles.

Functions of the HEF Glycoprotein During the Life Cycle

Research on ICV and IDV has concentrated mainly on its only glycoprotein HEF that combines the function of HA and NA of IAV and IBV, i.e., it possesses receptor-binding, receptor-destroying and membrane fusion activities. HEF (like HA) is a typical type 1

transmembrane protein with a short N-terminal, cleavable signal peptide, a long ectodomain, a transmembrane region and a very short, cytoplasmic tail which contains (in contrast to HA of Flu B) the longer acyl chain stearate. HEF is synthesized as a precursor HEF_0, which is cleaved into the N-terminal HEF_1 subunit and the membrane-anchored HEF_2 subunit. Although there is only 12% amino acid identity between HA and HEF, crystallography of their ectodomains revealed that the overall structures as well as the folding of individual domains are quite similar. Both glycoproteins form a mushroom-shaped trimer consisting of a membrane-near stalk (containing the regions involved in membrane fusion) and a globular head domain carrying the receptor-binding site. HEF contains an additional bulge located at the lower part of the globular domain that carries the receptor-destroying region that is not present in HA (**Fig. 3**).

Influenza C virus does not use sialic acid as its receptor on the target cell, but an acetylated derivative, namely 9-O-acetyl-N-acetylneuraminic acid. 9-O-Ac-Neu5Ac as the terminal sugar on both glycoproteins and glycolipids can be used as a receptor, regardless of whether it is attached to galactose via an α-2,3 or α-2,6 linkage. There is some evidence that the abundance of 9-O-Ac-Neu5Ac influences the tropism, since MDCK II cells (a standard cell line used for growth IAV and IBV) is resistant to ICV infection due to insufficient number of receptors. Instead, ICV is usually grown and plaque-titrated in another subline of Madin-Darby canine kidney cell, namely MDCK I cells.

The receptor binding site is located near the top of the globular head in a shallow groove surrounded by residues from four secondary structure elements: the 170-loop, 190-loop, 230-helix and 270-loop and thus exhibits a similar structure as the binding site for Neu5Ac in HA (**Fig. 3**). Interestingly, the crystal structure of HEF of IDV, which is otherwise almost identical to the structure of HEF of ICV, revealed a more open receptor-binding cavity, which may be the basis for the broader cell tropism of IDV. Some coronaviruses, such as the prototype member mouse hepatitis virus and human and bovine coronaviruses, contain a hemagglutinin esterase (HE) protein that also binds 9-O-Ac-Neu5Ac, but the ligand is bound in an opposite orientation.

Intrinsic temperature sensitivity is another peculiar feature of HEF of ICV that distinguishes it from HA. Folding of the protein in the endoplasmic reticulum (and hence cell surface expression) is less efficient at 37°C than at 33°C. Probably as a consequence, ICV grows to higher titers in cells cultured at 33°C compared with 37°C. This temperature sensitivity may be one of the factors that prevents the replication of ICV in the lungs that has a higher temperature compared with the upper respiratory tract. On the contrary, HEF of IDV exhibits an exceptional temperature and acid-stability and hence IDV is the most stable of the four types of influenza viruses, a feature that likely affects the tissue tropism and cross-species transmission of the virus.

Influenza C virus is proposed to enter cells via the endocytic pathway and to subsequently fuse with the membrane of the endosome. This process is also mediated by HEF and requires its proteolytic cleavage into HEF_1 and HEF_2 subunits and subsequent exposure to mildly acidic pH. Although the fusion mechanism of HEF has not been investigated, it is believed to be similar to HA where well-studied conformational changes catalyze membrane fusion. This assumption is based on the very similar structures of HA and HEF at neutral pH and especially the presence of a long central α-helix in HEF_2 and a smaller helix on the outside packed in an antiparallel fashion, which undergo large acid-induced conformational changes that are the main drivers for membrane fusion (**Fig. 3**). The long α-helix contains a predicted heptad-repeat, a characteristic element of most viral fusion proteins that interact in the low pH structure to form a stable 6-helix coiled-coil domain.

A notable difference between HA and HEF is the fusion peptide, a hydrophobic region at the N-terminus of the small HEF_2 subunit which contains hydrophobic, aromatic, but also a few negatively charged residues. It is fully conserved between ICV and IDV HEF (IFGIDDLI), but very different from the respective, strictly conserved amino acids of HA2 (GLFGAIAGFIE). In addition, while the fusion peptide of HA is completely buried in the interior of the molecule, the fusion peptide of HEF has a loop-like structure, which is partly exposed at the surface of the virus. HEF may therefore be regarded as having an internal fusion peptide, similar to virus fusion proteins that do not require cleavage activation.

HEF from both ICV and IDV possess a monobasic cleavage site (one arginine at the C-terminus of HEF_1) and it is in this respect similar to the HAs from mammalian and low pathogenic avian IAVs. The replication of these viruses is restricted to the site of virus infection, usually the respiratory tract. Interestingly, the amino acids surrounding the monobasic cleavage site of HEF differ between ICV and IDV. HEF from ICV contains three closely spaced basic residues at the C-terminus of HEF_1 (TVT*K*P*K*S*R*), whereas HEF_1 from IDV contains only two, widely spaced basic residues (*R*TLTPAT*R*). The molecular features of the cleavage site in viral glycoproteins determine which of the many trypsin-like serine proteases recognize the precursor protein and the expression of this protease determines whether a certain cell type produces infectious virus particles. Thus, differential cleavage activation of HEF may contribute to the differences in host range of ICV and IDV, i.e., human versus cattle.

The last step in the viral replication cycle is the release of virus particles from infected cells, which requires the removal of all putative receptors. In influenza A and B the receptor-destroying activity is carried out by the second viral glycoprotein, the neuraminidase NA, whereas HEF performs this activity for ICV. In accordance with its receptor binding specificity, HEF is not a neuraminidase, but an esterase that cleaves acetyl from the C9 position of terminal 9-O-Ac-Neu5Ac. The esterase activity, which belongs to the class of serine hydrolase, is included in an additional domain of HEF_1. The esterase domain is structurally the most conserved part of HEF; the position of the catalytic triad characteristic for serine hydrolases (serine 57, aspartic acid 356 and histidine 359) is identical in ICV and IDV HEFs, which exhibit 55% amino acid identity and an almost identical 3D-structure. The structure of the esterase site is also very similar between HEF and coronavirus HE and it has been proposed that the HE arose from Influenza C-like HEF by a lateral gene transfer of the middle part of the HEF gene encoding mainly the receptor-binding and esterase domains.

Epidemiology

Although influenza C virus infections occur primarily in a pattern of sporadic cases or in limited outbreaks, serological studies indicated that this virus is widely distributed around the world and that the majority of humans develop antibodies against the virus early in life, which prevent subsequent infections with ICV or mitigate the symptoms. According to a phylogenetic analysis of their HEF genes the existing strains are divided into six genetic and antigenic lineages. The average rate of nucleotide substitution in HEF has been calculated to be 4.9×10^{-4} substitutions/site/year, an order of magnitude slower compared to the corresponding rate for the HA gene of IAV. Only 40 residues ($\sim$5%) are not completely conserved through all lineages of HEF, the variable amino acids are mainly located in the globular head domain of HEF_1. Reassortment between viruses of different lineages occurred frequently, and newly emerged reassortants replaced previously circulating viruses and became epidemic strains. The primary host and reservoir of influenza C virus are humans, but antibodies against influenza C virus are present in dogs and especially in pigs. A number of influenza C virus strains were isolated from pigs in China and these strains could be experimentally transmitted from pig to pig.

Infection of humans by IDV has not been reported, but based on serological studies it is assumed that the virus circulates widely in cattle, especially in calves and the virus may spread to pigs and small ruminants (sheep and goat). Based on the comparison of the nucleotide sequence of the HEF gene, two distinct co-circulating lineages are currently described, which frequently reassort with each other, but not with ICV. HEF of IDV is also stable in evolution, but the substitution rate for HEF from IDV is 1.54×10^{-3} per site and year, and thus faster than in the HEF of ICV. The reason for the slow evolutionary rate of HEF from ICV is unknown. It may be either a low error rate of the polymerases and/or that HEF does not tolerate many amino acid changes without compromising its functionality.

Clinical Features and Pathogenesis

Influenza C virus usually causes inflammation of the upper respiratory tract, especially in children from one to six years of age. Clinical symptoms, such as cough, fever and malaise are typically mild. Occasionally the virus can spread to the lower respiratory tract, causing symptoms such as bronchitis and pneumonia, particularly in children younger than two years.

Experimental infection of pigs and cattle with IDV causes only mild symptoms (rhinitis and tracheitis) and virus replication is usually restricted to the upper respiratory tract. This is somewhat in conflict with the observation that IDV is associated with a more pronounced disease in the field and it was thus proposed that the manifestation of clinical symptoms requires coinfection with other respiratory viruses or bacteria that colonize the respiratory tract.

Diagnosis

Due to the usually mild clinical symptoms no rapid diagnostic tests are in place for ICV. These infections are usually identified in respiratory patient samples in retrospective studies using RT-PCR targeting the viral M or NP genes and/or virus isolation in tissue culture. Also, infection by IDV is mainly detected by RT-PCR analyzes of respiratory samples such as nasal and pharyngeal swabs or lung tissue.

Treatment and Prevention

Drugs developed against influenza A virus are not effective against ICV or IDV, since their target molecule is not present (neuraminidase inhibitors) or there are different amino acids at the binding site (M2 inhibitors). Since ICV is not thought to be a significant concern for human health, no vaccines have been developed. Prototype vaccines against IVD are currently under development.

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