Since their emergence as avian (1996) and zoonotic human pathogens (1997), H5N1 influenza viruses have spread to large parts of the world. Human infections remain sporadic, but they are associated with severe disease and high mortality (63% [241/382], per 30–4–08 [WHO, 2008]). High viral replication and an increased inflammatory response with cytokine dysregulation are thought to play central roles in the pathogenesis of human disease.

2. Pathogenesis

2.1. Clinical syndrome

Most human cases present as severe pneumonia with rapid progression to acute respiratory distress syndrome (ARDS). Patients show radiological evidence of pneumonia, sometimes with bilateral infiltration and collapse or consolidation. Complications associated with fatal outcome include ARDS and multi-organ failure. Occasionally, encephalitis has been observed. In recovered patients,
radiological evidence of lung damage may still be observed after several months (de Jong and Hien, 2006).

High nasopharyngeal viral loads were associated with fatal outcome (de Jong et al., 2006). Evidence of actively replicating H5N1 virus was found in the trachea and lower respiratory tract. In addition, viral RNA was demonstrated in autopsy samples of multiple non-respiratory organs including intestines, liver, spleen and brain, suggesting widespread viral dissemination (Abdel-Ghafar et al., 2008). In a pregnant woman, viral RNA and antigen was also detected in fetus and placenta (Gu et al., 2007). The isolation of H5N1 viruses from plasma suggests that the blood is a route of dissemination from primary infected (respiratory tract) sites to other organs. It remains unknown how H5N1 virus reaches the central nervous system: hematogenously through crossing of the blood–brain barrier or continuously by spread from peripheral nerve endings, as suggested by studies in cats and mice (Rimmelzwaan et al., 2006; Tanaka et al., 2003).

Patients with severe H5N1 disease often have lymphopenia, thrombocytopenia, and increased levels of serum aminotransferases (de Jong and Hien, 2006). Most examined patients have increased levels of chemokines (interferon [IFN]-inducible protein 10 [IP-10]), monokine induced by IFN-γ [MIG], monocyte chemotactic protein-1 [MCP-1], Interleukin [IL]-8) and pro- and anti-inflammatory cytokines (IL6, IFN-γ, IL-10), the levels of most of which were positively correlated with both nasopharyngeal virus loads and fatal outcome (de Jong et al., 2006). In vitro experiments in macrophages and respiratory epithelial cells have shown that H5N1 viruses may induce cytokine expression to a larger extent than human seasonal influenza viruses (Chan et al., 2005; Cheung et al., 2002). This observed high production of cytokines may be explained by strong activation of the p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway by H5N1 (Lee et al., 2005).

2.2. Hemagglutinin (HA)

The hemagglutinin (HA) plays a role in viral entry into host cells and determines host specificity of influenza viruses. HA binds to glycan receptors terminated by a sialic acid (SA) linked to a galactose residue. Traditionally it was thought that avian hosts have 2,3 linked sialic acids (SA2,3-Gal) in their respiratory and gastrointestinal tract, while humans express SA2,6-Gal. Likewise, avian and human viruses preferentially bind to the corresponding sialic acids of their hosts. Receptor specificity may change by a small number of amino acid changes in the receptor-binding pocket of HA (Naeve et al., 1984).

Recent studies revealed expression of SA2,3-Gal in human respiratory epithelial cells: cells of the upper respiratory tract (nasal mucosa, paranasal sinuses, pharynx, and trachea) express mainly SA2,6-Gal, whereas cells in the lower respiratory tract (type II pneumocytes and non-ciliated cuboidal epithelial cells) express both SA2,6- and 2,3-Gal. SA2,3-Gal bearing cells in the lower respiratory tract are believed to act as primary target cells for H5N1 virus infections. The predominance of these receptors in the lung may explain the propensity of H5N1 viruses to cause pneumonia in humans as well as the difficulty in transmission of this virus from birds and between humans (Shinya et al., 2006; van Riel et al., 2006). Compared to adults, respiratory cells from children seem to express more SA2,3-Gal, suggesting – in accordance with epidemiological data – that they may be more susceptible to H5N1 infection (Nicholls et al., 2007a,b). However, data from glycan arrays show that the structural topology of the glycan also plays an important part besides the linkage of the terminal sialic acid (Chandrasekaran et al., 2008) and that viruses...
may bind to a whole range of other glycans apart from SA α2,3 and α2,6-Gal (Stevens et al., 2006), demonstrating that influenza receptor specificity is (far) more complex than previously recognized.

2.3. The polymerases

Changes in the viral polymerase complex, particularly in PB2, seem essential for adaptation of avian influenza viruses to efficient replication and pathogenicity in mammalian hosts. Specifically, the presence of a lysine (Lys) instead of a glutamic acid (Glu) at position 627 of PB2 has been identified as an important determinant of host range (Subbarao et al., 1993). Furthermore, in vitro studies have shown that viruses with Lys627 replicate efficiently at lower temperatures (33 °C), providing opportunities for efficient growth in the upper respiratory tract of mammalian hosts. Accordingly, viruses containing Lys627 replicate to higher titres in nasal turbinates of mice than viruses with Glu627 (Neumann et al., 2007). While mouse models suggest that Lys627 in H5N1 PB2 is a major determinant of high pathogenicity in mammals, a clear association with poor clinical outcome in humans has not been found.

Interestingly, in a series of Vietnamese H5N1 patients, most viruses lacking Lys627 harboured an alternative change at position 701 (Asp to Asn) which has also been linked to adaptation of avian viruses to mammalian hosts (de Jong et al., 2006). In mice, H5N1 viruses lacking an Asn701 residue were unable to replicate and cause disease (Li et al., 2005). In H7N7 viruses, the presence of Asn at position 701 was shown to be involved in binding to mammalian importin 1α, thereby enabling more efficient transport of the replication machinery to the nucleus of the host cell (Gabriel et al., 2008).

While specific changes in PB2 thus seem important for efficient replication and pathogenicity in mammals, changes in other polymerase genes likely also play a role. This is illustrated by reverse genetics experiments showing more pronounced virulence changes when all three polymerase genes from a highly virulent virus, rather than single genes, were introduced in a non-pathogenic virus (Salomon et al., 2006).

2.4. The PB1-F2 protein

The recently discovered PB1-F2 protein is translated from an alternate reading frame of the PB1 gene segment. PB1-F2 causes apoptosis in macrophages, reducing their ability to induce an immune response and therefore delaying viral clearance (Coleman, 2007). This reading frame is also present in H5N1, and mutations cause virulence changes (Conenello et al., 2007). The exact role in pathogenicity has yet to be determined.

2.5. The NS1 protein

The NS1 protein is the only nonstructural protein of influenza viruses. This protein plays an important role in the pathogenicity of influenza viruses by protecting the virus from the antiviral effects of the host IFN responses. NS1 exerts its IFN antagonism by binding to dsRNA and to the cellular RNA helicase retinoic acid inducible gene 1 (RIG-1), an upstream regulatory component of the IFN production cascade (Mibayashi et al., 2007).

In highly pathogenic H5N1 viruses, the NS1 gene is also implicated in causing cytokine dysregulation, which is thought to play an important role in H5N1 disease pathogenesis in mammals, including humans. Compared to human H1N1 and H3N2 viruses, the NS1 gene of H5N1 viruses or its product is a potent inducer of proinflammatory cytokines in vitro, particularly TNF-α (Cheung et al., 2002). Contemporary human H1N1 virus carrying the NS1 of a highly pathogenic H5N1 virus isolated during the 1997 Hong Kong outbreak induced high pulmonary concentrations of pro-inflammatory cytokines and prolonged viral shedding in pigs (See et al., 2002). While this effect was linked to a specific amino acid change in NS1 (Asp92Glu), this change has not been observed in current H5N1 strains.

Whole genome sequencing revealed a PDZ domain ligand (PL) in the NS1 carboxy terminus of influenza virus. Proteins that contain PDZ domains play important roles in many key signaling pathways. Avian PL signatures from H5N1 and H1N1 were shown in vitro to bind human PDZ domains more efficiently than human H2N2 and H3N2 viruses, possibly disrupting more cellular processes (Obenauer et al., 2006). The exact consequences of these findings on the virulence of influenza viruses in general and of H5N1 viruses in particular have yet to be determined.

3. Treatment

At present, two classes of drugs are in use for the treatment of influenza virus infections: the adamantanes (amantadine and rimantadine), targeting the M2 ion channel of influenza A viruses, and the neuraminidase inhibitors (zanamivir and oseltamivir). Susceptibility to adamantanes is varying in current H5N1 viruses. Adamantane resistance rates are high in certain genetic clades of H5N1 viruses, which may be explained by the use of amantadine in poultry farming (Abdel-Ghafar et al., 2008; Deyde et al., 2007; He et al., 2008). H5N1 viruses are usually susceptible to neuraminidase inhibitors, although up to 30-fold differences in in vitro 50% inhibitory concentrations of oseltamivir are observed between different genetic clades of current H5N1 viruses (McKimm-Breschkin et al., 2007). Development of high levels of resistance of H5N1 virus can occur during treatment with oseltamivir and is associated with treatment failure (de Jong et al., 2005). While oseltamivir resistant viruses remain susceptible to zanamivir (Wetherall et al., 2003), the currently available locally acting inhaled formulation of this drug limits its potential use in human H5N1 infections in view of the systemic nature of this disease. Parenteral formulations of neuraminidase inhibitors as well as novel drugs with alternative targets, such as the viral polymerase, are desirable and currently under clinical development. The potential use of combination therapy to enhance antiviral efficacy and prevent the development of drug resistance may need consideration.

The benefits of antiviral treatment are highly dependent on the timing of treatment. Early installment of treatment clearly seems associated with improved outcome of
human H5N1 infections (Abdel-Ghafar et al., 2008), likely due to prevention of irreversible tissue damage by the virus and the ensuing host immune response. At present there is insufficient evidence suggesting a role of specific immunomodulatory agents in the treatment of H5N1 infections. Studies in knock-out mice revealed conflicting results showing milder disease courses in TNF receptor-deficient mice in one study and unchanged outcomes of TNF or TNF receptor-deficient mice in another (Salomon et al., 2007; Szezetter et al., 2007). The latter study also showed no beneficial effects of glucocorticoid treatment of mice but combined antiviral and steroid treatment was not evaluated (Salomon et al., 2007). Observational data in humans showed higher, rather than lower mortality rates in H5N1-infected patients receiving steroids in addition to oseltamivir suggesting there is no role of steroids in the treatment of human H5N1 diseases (Abdel-Ghafar et al., 2008).

4. Conclusion

Knowledge gained from clinical observations and experimental research suggest that high viral replication efficiency, broad tissue tropism and an intense inflammatory response play critical roles in the pathogenesis of H5N1 influenza. Clinical management should be focused on early and effective suppression of viral replication. The potential role of immunomodulatory intervention remains unclear and requires further unraveling of the molecular and cellular mechanisms underlying H5N1 pathogenesis to afford a rational approach.

References


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