The fate of influenza A virus after infection of human macrophages and dendritic cells

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Introduction

Influenza viruses belong to the family Orthomyxoviridae of enveloped viruses and are an important cause of respiratory infections worldwide. These negative-sense, ssRNA viruses can be classified into three distinct types (A, B and C) based on antigenically distinct internal proteins. Type A influenza virus (IAV) is the major aetiological agent of epidemics and pandemics in humans, and is also capable of infecting birds, swine, horses and other mammals.

In humans, IAV infection is predominantly restricted to the respiratory tract (Taubenberger & Morens, 2008). IAV infection of respiratory epithelial cells is initiated following recognition of cell-surface sialic acid by the viral haemagglutinin (HA) protein (Skehel & Wiley, 2000; Wiley & Skehel, 1987). In general, human IAV strains preferentially bind to terminal sialic acids linked to the underlying galactose residue in an α-2,6 conformation, which is abundant on cells in the human upper respiratory tract (Connor et al., 1994; Ibricevic et al., 2006; Rogers & D’Souza, 1989; Rogers & Paulson, 1983; Thompson et al., 2006). In addition to HA-sialic acid binding, it is likely that interactions with other, as-yet-unidentified, cell surface receptors facilitate virus entry into epithelial cells (Chu & Whittaker, 2004; Oshansky et al., 2011; Rapoport et al., 2006; Thompson et al., 2006). IAV infection of epithelial cells is considered to be ‘productive’ as new infectious virions are released from infected cells. In contrast, the outcome of IAV infection of host immune cells, specifically macrophages (MΦ) and dendritic cells (DC), is less clear.

Alveolar MΦ and DC are also among the first cells in the respiratory tract to detect and respond to IAV, where they play a pivotal role in mounting effective innate and adaptive responses (Grayson & Holtzman, 2007; Kreijtz et al., 2011; McGill et al., 2009; Summerfield & McCullough, 2009). As for epithelial cells, binding of IAV HA to sialic acid concentrates virus at the cell surface and this may promote subsequent interactions with other receptors, such as C-type lectin receptors (Londrigan et al., 2012). Several studies have reported that IAV replicates productively in MΦ and DC (Hoeve et al., 2012; Perrone et al., 2008; van Riel et al., 2011; Yu et al., 2011). However, others have described these infections as ‘abortive’ (Bender et al., 1998; Ioannidis et al., 2012; Reading et al., 2000; Rodgers & Mims, 1981, 1982; Tate et al., 2010; Wells et al., 1978). For the purposes of this review, we define an abortive infection as one where infectious viral particles are not released from infected cells. Abortive infections are characterized by early signs of viral replication, including the detection of viral gene transcription and/or the production of viral proteins demonstrated in the aforementioned studies. However, at some point in the replication cycle viral replication is blocked, such that no virions are produced. The degree to which the IAV replication cycle extends through to viral assembly and release remains largely undetermined.

Understanding the fate of IAV following infection of MΦ and DC will enhance our understanding of the role of these cells in controlling (or facilitating) virus spread as well as disease severity. Thus, this review critically examines the outcomes of IAV infection of MΦ and DC by different IAV subtypes and strains. Moreover, we discuss the role that particular host and viral factors may play in modulating IAV...
infection of MΦ and DC, and the in vivo consequences of productive versus abortive infection for IAV pathogenesis.

IAV subtypes and strains

The IAV genome comprises eight RNA segments that encode at least 11 proteins, including the HA and neuraminidase (NA) glycoproteins that protrude from the surface of the virion. IAV strains are divided into 16 HA and nine NA subtypes based on the antigenically and structurally distinct HA and NA that they bear (Obenauer et al., 2006). During the past century, IAV pandemics have occurred in 1918, 1957 and 1968 following the emergence of H1N1, H2N2 and H3N2 subtypes, respectively. The first pandemic of this century occurred in 2009 and arose from an IAV of swine origin (Smith et al., 2009). Human infections with avian-derived IAV, including H5N1, H7N7 and H9N2, have also been described. However, to date these subtypes have not acquired the ability to transmit efficiently in the human population (Suzuki, 2005). Various IAV subtypes are compared and described in detail below.

H1N1

In 1918, H1N1 IAV [termed 1918 pandemic (pdm) H1N1] gave rise to the ‘Spanish flu’ pandemic. This is the most lethal pandemic on record with an estimated 50 million fatalities worldwide (Johnson & Mueller, 2002). Whilst the pandemic abated in 1919, H1N1 strains continued to circulate in the human population until 1957. In 1977, H1N1 strains re-emerged and with seasonal variation continued to circulate until recently, when they were replaced by a novel H1N1 strain of swine origin (2009 pdm H1N1) associated with the latest pandemic (‘swine flu’) in 2009 (http://www.who.int/influenza). Compared with seasonal H1N1 strains, 1918 pdm H1N1 viruses are highly pathogenic in non-human primate and murine models of infection (Baskin et al., 2009; Perrone et al., 2008; Tumpey et al., 2005a, b). In contrast, immunopathological studies using tissues from IAV-infected humans (Calore et al., 2011; Capelozzi et al., 2010; Gill et al., 2010; Rosen et al., 2010) or experimentally infected animals (Brookes et al., 2010; Kang et al., 2011; Khatri et al., 2010; Lange et al., 2009; Memoli et al., 2009; Safronetz et al., 2011; Smith et al., 2011) indicate that 2009 pdm H1N1 viruses were of modest virulence. In addition to seasonal H1N1 strains, A/Puerto Rico/8/34 (PR8) and A/WSN/33 (WSN) are commonly used H1N1 viruses derived by serial passage through mice (Fislova et al., 2009; Ginsberg & Horsfall, 1952; Tate et al., 2010, 2011). These and other mouse-adapted IAV have been used extensively to provide insights regarding pathogenesis, disease and immunity to IAV in the mouse model.

H3N2

In 1968, H3N2 IAV first appeared in the human population and gave rise to the ‘Hong Kong flu’ pandemic, and resulted in up to 2 million fatalities worldwide (Guan et al., 2010). Seasonal H3N2 strains continue to circulate in humans today. Compared with 1918 pdm H1N1 IAV and highly pathogenic H5N1 (see below), H3N2 strains display only modest virulence in a variety of animal models of infection (reviewed by Guarner & Falcon-Escobedo, 2009; Peiris et al., 2009).

H5N1

Human infections with H5N1 avian influenza viruses (AIV) were first reported in 1997 during a poultry outbreak in Hong Kong (Claas et al., 1998; Korteweg & Gu, 2008; To et al., 2001) and have since been found to have a case-fatality rate of approximately 60% (Abdel-Ghafar et al., 2008). The pathogenic nature of H5N1 can be partially attributed to its ability to spread systemically in humans and other mammals, compared with other IAV strains that are usually restricted to the respiratory tract. Using a ferret model, Schrauwen et al. (2012) demonstrated that the multi-basic cleavage site in the viral HA of H5N1 was a critical determinant of systemic spread. The exaggerated inflammatory response and ‘cytokine storm’ observed in H5N1 patients is also thought to contribute to the pathogenicity of the virus, although treatment with anti-inflammatory agents may not necessarily ameliorate disease (Fedson, 2009).

The role of MΦ in IAV infection

In general terms, MΦ are long-lived terminally differentiated tissue resident cells capable of limited cellular division. They are considered to be sentinel phagocytes of the innate immune system involved in clearing pathogens, apoptotic cells and in antigen presentation to T-cells. Chemokines and cytokines produced by MΦ in response to a broad range of stimuli act to modulate inflammation, promote clearance of infectious agents and to restore tissue homeostasis. In humans, many different macrophage (MΦ) subsets have been described. To date, in vitro studies examining interactions between IAV and human MΦ have largely focused on alveolar MΦ (AMΦ) and MΦ derived from peripheral blood mononuclear cells (PBMCs). In vivo, AMΦ are long-lived residential cells of the lung which represent one of the first ‘responder cells’ to invading pathogens (Schneberger et al., 2011). In the absence of infection, AMΦ maintain lung homeostasis and display an anti-inflammatory or ‘alternatively activated’ phenotype (Holt, 1986). Human AMΦ express α-2,3- and α-2,6-linked sialic acid (Yu et al., 2011) as well as C-type lectin receptors such as MΦ mannose receptor (MMR) (Stephenson & Shepherd, 1987) and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) (Soilleux et al., 2002). Another C-type lectin, the MΦ galactose-like lectin (MGL), is expressed by both human monocyte-derived MΦ (MDMΦ) (van Vliet et al., 2006a, 2008) and murine AMΦ (Imai et al., 1995), although expression of MGL by human AMΦ is yet to be reported. Clearly, human AMΦ are particularly relevant when studying responses to IAV and other respiratory pathogens, yet obtaining sufficient numbers can be challenging as
AMΦ must be isolated from bronchoalveolar lavage fluids for use in experimental studies.

In contrast, MDMΦ from PBMCs are more readily available, typically being generated by culturing CD14⁺ monocytes in vitro. Often, MDMΦ are cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) to mimic the abundant levels of this cytokine in the lung, and hence provide a more accurate model for AMΦ. Like AMΦ, MDMΦ display α-2,3- and α-2,6-linked sialic acid (Sakabe et al., 2011; Yu et al., 2011) and express DC-SIGN (Chehimi et al., 2003) as well as MMR (Nguyen & Hildreth, 2003). MDMΦ also express MGL, although the levels vary with the extent of differentiation (Higashi et al., 2002). However, whilst some phenotypic aspects of MDMΦ resemble those of AMΦ, there are marked differences between AMΦ and MDMΦ in their responses to IAV infection (van Riel et al., 2011; Yu et al., 2011). Therefore, responses of MDMΦ to IAV may not necessarily reflect responses of AMΦ and vice versa. Indeed, even in the presence of GM-CSF the cell subset-dependent differences in IAV infection cannot be overcome (van Riel et al., 2011). Both cell types remain pertinent to studying respiratory tract infections, as MDMΦ were proposed to represent MΦ recently recruited into the lungs, whereas AMΦ are more representative of the residential MΦ population (van Riel et al., 2011). Thus, for the purposes of this review we will consider AMΦ and MDMΦ as two distinct cell types, potentially modelling two different in vitro cell subsets.

**Infection of MΦ with seasonal IAVs**

Seasonal IAV strains (H1N1 and H3N2) readily infect human MDMΦ (Hoeve et al., 2012; Mok et al., 2007; Perrone et al., 2008; van Riel et al., 2011; Yu et al., 2011). Most studies report productive infection of human MDMΦ by seasonal IAV (Hoeve et al., 2012; Mok et al., 2007; Perrone et al., 2008; van Riel et al., 2011; Yu et al., 2011). However, Friesenhagen et al. (2012) recently demonstrated that under certain conditions PR8/34 infection of MDMΦ resulted in abortive infection.

AMΦ are also infected by seasonal IAV strains (Rodgers & Mims, 1982; van Riel et al., 2011; Yu et al., 2011) although there is conflicting evidence as to whether AMΦ and MDMΦ are equally susceptible to the early stages of infection (Rodgers & Mims, 1982; van Riel et al., 2011; Yu et al., 2011). This could reflect differences in the virus strains used between studies, in conjunction with variations in the expression patterns of cell surface receptors by different MΦ subsets. Regardless, infection of AMΦ with seasonal IAV is not productive (Rodgers & Mims, 1982; van Riel et al., 2011; Yu et al., 2011). Infection of mouse AMΦ by seasonal IAV is also abortive (Perrone et al., 2008; Reading et al., 2000; Rodgers & Mims, 1981; Wells et al., 1978). At present, it remains unclear how productive infection of AMΦ by seasonal IAV is impaired. However, detection of viral nucleoprotein (NP) in infected cells (van Riel et al., 2011; Yu et al., 2011) suggests a post-translational block during the assembly or release of virions.

**Infection of MΦ by 2009 pdm H1N1 IAV**

The 2009 pdm H1N1 strain A/NL/602/09 displayed a very low infection rate of both MDMΦ and AMΦ (van Riel et al., 2011). Accordingly, there was no significant viral production from either cell subset (van Riel et al., 2011). This is a somewhat unexpected observation, as early pandemic viruses display a dual receptor specificity (Childs et al., 2009). Given the presence of both α-2,3- and α-2,6-linked sialic acid on MDMΦ and AMΦ (Sakabe et al., 2011; Yu et al., 2011), 2009 pdm H1N1 would be expected to infect both cell types. The limited infection rate may suggest that alternative receptors are particularly important for entry of 2009 pdm H1N1 viruses into human MΦ. In a separate study, Sakabe et al. (2011) reported limited infectious virus in MDMΦ supernatants taken 12–36 h after incubation with A/California/04/2009, and levels were significantly lower than those obtained using seasonal H1N1 strains. However, as the level of infection was not assessed in this particular study, it remains unclear if a limited ability to infect human MΦ is true of all 2009 pdm H1N1 strains.

**Infection of MΦ by H5N1 and 1918 pdm H1N1 IAV**

The ability of H5N1 IAV to infect and replicate productively in human MΦ is controversial. Numerous studies have demonstrated that H5N1 strains infect MDMΦ productively (Perrone et al., 2008; Sakabe et al., 2011; van Riel et al., 2011; Yu et al., 2011). However, it remains uncertain if infection and subsequent virus release is more or less efficient compared to seasonal or pdm H1N1 (Mok et al., 2007; Perrone et al., 2008; Sakabe et al., 2011; van Riel et al., 2011; Yu et al., 2011). Moreover, Friesenhagen et al. (2012) recently demonstrated that infection of MDMΦ with the highly pathogenic KAN-I (H5N1) and FPV (H7N7) strains was not productive. There is also discordant evidence as to whether H5N1 infection is productive in AMΦ (van Riel et al., 2011; Yu et al., 2011) and it remains unclear if H5N1 viruses display an increased or equivalent infection rate of AMΦ compared to seasonal strains (van Riel et al., 2011; Yu et al., 2011).

There are a limited number of reports describing the ability of the 1918 pdm H1N1 virus to infect human MΦ. Perrone et al. (2008) showed that human MDMΦ were productively infected by a reconstructed 1918 pdm H1N1 strain. However, the ability of the 1918 pdm H1N1 virus to productively infect human AMΦ, to the best of our knowledge, has yet to be reported. Of interest, MΦ isolated from mouse lung (interstitial and AMΦ) supported only limited productive infection of 1918 pdm H1N1 when infected in vitro; however, significantly more virus was shed from ex vivo cultured MΦ isolated from the lungs of 1918 pdm H1N1-infected mice (Perrone et al., 2008).
Impact of IAV infection of MΦ on disease pathogenesis

Taken together, the studies described above suggest that virus subtype/strain differences, as well as MΦ subset-dependent differences, are likely to influence the outcome of IAV infection of MΦ (see Table 1). These studies emphasize the need for a systematic approach to this research, where a large panel of different IAV strains would be tested for their ability to infect different MΦ subsets within the one study. Differences in infection rate and/or infection outcome may have important consequences for the pathogenesis of particular virus strains in vivo. Thus, the inability of seasonal and 2009 pdm H1N1 strains to productively infect AMΦ would be consistent with a critical role in host defence, where the failure of these cells to contribute to productive viral amplification (at least in the early stages of infection) may lead to a reduction in the severity of infection by these strains. Assuming that the MDMΦ model newly recruited MΦ to the lung, their ability to support productive viral replication (at least in the majority of in vitro studies described) may represent a necessary 'trade off' later in infection when they are necessary to elicit innate and adaptive immune responses to overcome infection (see Fig. 1a).

As discussed, the ability of AMΦ to support productive replication of H5N1 and to mount innate antiviral responses differs between studies. Productive infection of AMΦ with A/Hong Kong/483/97 (H5N1) resulted in a pronounced pro-inflammatory response (Yu et al., 2011). However, non-productive infection of AMΦ with Vietnam/04 (H5N1) did not result in the production of the archetypal pro-inflammatory cytokine tumour necrosis factor alpha (van Riel et al., 2011). Moreover, although the study was performed using MDMΦ, Friesenhagen et al. (2012) showed that not only was the H5N1 strain KAN-1 unable to productively infect human MΦ, but it also actively suppressed inflammasome activation and pro-inflammatory cytokine responses. These data may suggest that a greater pro-inflammatory response is elicited when IAV productively (rather than abortively) infects AMΦ. Therefore, one possible scenario is that the ability of at least some H5N1 viruses to productively infect AMΦ may result in a higher viral load, an increased pro-inflammatory response and increased immunopathology relative to seasonal viruses (see Fig. 1b). This model is consistent with the high viral load and hyper-cytokinemia observed in fatal H5N1 cases (de Jong et al., 2006).

The role of DC in IAV infection

DC are specialized antigen-presenting cells designed to process and present foreign antigens to other cells of the immune system. They represent an important link between the innate and adaptive immune response (Grayson & Holtzman, 2007). DC are located in most peripheral tissues and, upon activation, move to secondary lymphoid tissues for interaction with T-cells during initiation of adaptive responses. In contrast to the numerous studies addressing the interactions between IAV with human MΦ, there are limited studies investigating how human DC respond to IAV. This may reflect, in part, the difficulties associated with translating what is known regarding DC responses in murine models of IAV infection to human immunology and disease. For example, unlike murine DC, human DC do not express CD8 on the cell surface, complicating inter-species comparisons of DC subsets (Shortman & Liu, 2002). Difficulties accessing and obtaining human lung samples also limit studies assessing the ability of IAV to infect human lung DC. Instead, the most common source of human DC is from the blood of healthy volunteers (Shortman & Liu, 2002). Myeloid DC (mDC; CD11c+ or CD141+) and plasmacytoid DC (pDC; CD303+) can be isolated directly from the blood. Alternatively, DC can be differentiated from blood monocytes in the presence of GM-CSF and IL-4. These ‘immature’ DC are similar to those found in peripheral tissues and can be further differentiated into mature (CD14-CD38+CD86+ surfaceMHCIIhi) DC by

Table 1. Infection of human MΦ with IAV

<table>
<thead>
<tr>
<th>IAV Type</th>
<th>Able to infect MDMΦs?</th>
<th>Productive in MDMΦs?</th>
<th>Able to infect AMΦs?</th>
<th>Productive in AMΦs?</th>
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<tr>
<td>Seasonal IAV</td>
<td>✓ Friesenhagen et al. (2012); Hoeve et al. (2012); Mok et al. (2007); Perrone et al. (2008); van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ Friesenhagen et al. (2012); Hoeve et al. (2012); Mok et al. (2007); Perrone et al. (2008); van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ Rodgers &amp; Mims (1982); van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ Rodgers &amp; Mims (1982); van Riel et al. (2011); Yu et al. (2011)</td>
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<tr>
<td>2009 pdm H1N1 H5N1</td>
<td>✓ van Riel et al. (2011); Friesenhagen et al. (2012); Perrone et al. (2008); Sakabe et al. (2011); van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ van Riel et al. (2011); Friesenhagen et al. (2012); Perrone et al. (2008); Sakabe et al. (2011); van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ van Riel et al. (2011); Yu et al. (2011)</td>
<td>✓ van Riel et al. (2011); Yu et al. (2011)</td>
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<td>1918 pdm H1N1</td>
<td>✓ Perrone et al. (2008)</td>
<td>✓ Perrone et al. (2008)</td>
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ND, Not done.
exposure to pro-inflammatory cytokines or selected pattern-associated molecular patterns (e.g. LPS) (Shortman & Liu, 2002). Human monocyte-derived DC (MDDC) express both α-2,3- and α-2,6-linked-sialic acid (Thitithanyanont et al., 2007) along with varying levels of C-type lectin receptors including MMR (Engering et al., 1997), DC-SIGN (Geijtenbeek et al., 2000; Soilleux et al., 2002) and MGL (van Vliet et al., 2006a, b, 2008). At present, it remains unclear how closely MDDC model those DC that reside in the lungs and nasopharynx, and this is one caveat in interpreting any data derived using these cells. Nevertheless, blood monocytes provide an easily accessible source of DC to study IAV infection.

Infection of DC with seasonal IAVs

Early studies using PR8/34 showed that IAV readily infected immature human MDDC (Bender et al., 1998). However, this infection resulted in the production of minimal, if any, infectious virus (Bender et al., 1998). Similar conclusions were drawn following infection of immature MDDC by a seasonal H1N1 strain (Perrone et al., 2008). Interestingly, Cella et al. (1999) demonstrated that whilst immature MDDC could be infected with PR8/34, mature MDDC were almost completely resistant to infection. This suggests that the susceptibility of human DC to the early stages of IAV infection may be affected by the stage of DC maturation or differentiation. Indeed, downregulation of putative IAV cell surface receptors (MMR, MGL and DC-SIGN) on MDDC after LPS-maturation has been demonstrated in vitro (van Vliet et al., 2006b).

**Infection of DCs by 2009 pdm H1N1 IAV**

Osterlund et al. (2010) demonstrated that immature human MDDC were readily infected with 2009 pdm IAV strain A/Finnland/553/2009. Replication (as measured by viral M1 gene expression over time) was similar between seasonal H1N1/H3N2 strains and A/Finnland/553/2009. This study did not address whether the infection was productive or abortive, although the 2009 pdm H1N1 virus did not induce cytopathic effects in DC as rapidly as seasonal IAV, suggesting that viral subtype-dependent differences may exist during DC infection.

**Infection of DCs by H5N1 and 1918 pdm H1N1 IAV**

Productive infection of human MDDC by AIV H5N1 has been reported (Perrone et al., 2008; Thitithanyanont et al., 2007). Perrone et al. (2008) compared the ability of seasonal H1N1, reconstructed 1918 pdm H1N1 and both highly pathogenic AIV (HPAIV) and low pathogenic AIV (LPAIV) H5N1 strains to infect mouse and human DC. In these studies, only HPAIV H5N1 showed evidence of productive

![Fig. 1. Consequences of IAV infection of human MΦ. (a) Infection of AMΦ with seasonal H1N1 or 2009 pdm H1N1 is abortive (i.e. no infectious virus is produced) and thus helps limit the severity of the viral infection by serving as a ‘dead end’ route of virus infection. However, MΦ subsequently recruited to the lung (represented by MDMΦ) are likely to support active viral replication. (b) Infection with some strains of H5N1 may lead to productive replication in both residential MΦ and newly recruited MΦ. This may play a role in the enhanced disease severity associated with these strains, as increased viral production may in turn lead to increased inflammation and immunopathology.](http://vir.sgmjournals.org)
infection in human MDDC and primary mouse lung DC. Furthermore, Thitithanyanont et al. (2007) reported that MDDC produced approximately the same number of HPAIV H5N1 virions as infected primary human bronchial and tracheal epithelial cells. This is a striking observation, given that epithelial cells are thought to be the primary target of IAV infection and leukocyte infection is typically considered less efficient. H5N1 virus also induced a stronger cytopathic effect in MDDC compared with seasonal H3N2 virus (Thitithanyanont et al., 2007). Interestingly, whilst mDC purified directly from the blood were permissive to infection (albeit an abortive infection), purified pDC were completely resistant to infection (Smed-Sörensen et al., 2012; Thitithanyanont et al., 2007). Similar to the inability of mature MDDC to be infected with seasonal IAV strains (Cella et al., 1999), in this instance resistance to infection was associated with the ability of pDC to produce large amounts of type I interferon (IFN) (Thitithanyanont et al., 2007). Therefore, the aforementioned studies may suggest that virulent IAV strains may be more adept at replication in certain subsets of DC. However, further studies are required to determine whether lung DC behave similarly to MDDC.

Impact of IAV infection of DC on disease pathogenesis

Given the limited number of studies that have investigated the fate of IAV following infection of human DC (see Table 2), it is difficult to speculate about the in vivo consequences of a productive versus abortive infection of DC. Perhaps the most relevant scenario pertains to the infection of DC by H5N1 strains. While IAV-infected MΦ remain in the airways, IAV-infected DC can traffic to the lymph nodes to present antigen. The ability of DC to produce not only infectious virus, but also infectious virus in large quantities, combined with their mobility, may then contribute to the systemic spread of IAV (see Fig. 2). This is of particular relevance to H5N1 strains that, due to the presence of the multi-basic cleavage site, are able to replicate outside the respiratory tract (Schrauwen et al., 2012). Moreover, H5N1-infected peripheral blood DC can pass virus (H5N1 pseudotyped and reverse genetics particles) to epithelial cells, thereby promoting infection of susceptible cells in trans (Wang et al., 2008). Large amounts of viable H5N1 virus have been detected in the blood of infected patients (de Jong et al., 2006). This also implies that DC may play an important role in the systemic spread and dissemination of some highly pathogenic IAV strains. This theory is corroborated by studies in animals, where Molthedo et al. (2011) describe multi-cycle replication of virulent mouse-adapted strains in migratory lung DC isolated from the lymph nodes of infected mice. Interestingly, clinical evidence suggests that fatal cases of H5N1 in humans are associated with severe lymphopenia (de Jong et al., 2006). It is therefore tempting to speculate that IAV-infected DC produce infectious virus in the lymph nodes; this then infects the surrounding B- and T-cells and triggers lymphocyte apoptosis (see Fig. 2b). Whilst Thitithanyanont et al. (2007) showed that B-cells and αβ T-cells were resistant to H5N1 infection in vitro, whether or not this recapitulates the in vivo situation in the lymph node remains to be determined.

Viral and cellular factors that may control productive infection of MΦ and DC by IAV

It is well established that IAV infection of respiratory epithelial cells results in a productive infection, whereby newly synthesized infectious virions are released from infected cells. However, there are distinct differences in the capacity of immune cells, namely airway MΦ and DC, to support productive IAV infection when compared with epithelial cells. This review has highlighted the complexity of IAV infection of MΦ and DC, where productive or abortive infection occurs depending upon the IAV subtype or strain and the specific MΦ or DC subset being studied (Tables 1 and 2). Thus, future studies must take these differences into account so that a consensus can be reached regarding the fate of IAV following infection of MΦ and DC. We defined an abortive infection as a ‘dead end’ route of infection, such that newly synthesized infectious virions are not released from infected cells. However, the precise point to which the IAV replication extends during abortive infection of MΦ and DC is not clear. Recently, IAV infection of mouse bone-marrow-derived DC was shown to be abortive, despite transcription of each gene segment of the viral genome and production of HA and NP viral proteins by infected cells (Ioannidis et al., 2012). This suggests the block is unlikely to have occurred during the early stages of infection. Indeed, these authors used electron microscopy to show that assembly of viral particles was defective in these cells. Other studies corroborate production of at least some viral proteins during abortive infection of murine AMΦ (Rodgers & Mims, 1981), human AMΦ (Rodgers & Mims, 1982) and human MDDC (Bender et al., 1998) by IAV. Furthermore, productive replication by some IAV strains, namely HPAIV H5N1, indicates that MΦ and DC possess the cellular machinery required for the assembly and budding of viral particles, but that the ability of different strains to use these may vary. Dysregulated production, processing and/or stability of particular viral proteins in MΦ and DC could also result in abortive infection as has been reported during IAV infection of Vero cells, where viral HA glycoprotein maturation and transport to the plasma membrane was disturbed (Lau & Scholtissek, 1995).

Alternatively, intrinsic antiviral factor/s expressed by some MΦ and DC may act to inhibit virus replication, assembly and/or release. There is considerable variation in the ability of certain MΦ and DC subsets to support human immunodeficiency virus type 1 (HIV-1) infection and replication (Bergamaschi & Pancino, 2010; Manel et al., 2010) and recently SAM domain and HD domain-containing protein 1 was identified as a dendritic and myeloid cell-specific restriction factor for HIV-1 (Lagayette et al., 2011). Several host antiviral restriction factors have been recently identified for IAV in what is a key emerging area of research (reviewed by...
Yan & Chen, 2012). Specifically, IAV cytosolic entry may be inhibited by interferon-induced transmembrane protein 3 (IFITM3) (Brass et al., 2009; Feeley et al., 2011), and enhanced pathogenesis of IAV has now been associated with loss of IFITM3 function in both mice and humans (Everitt et al., 2012). In addition, MxA can block IAV RNA transcription (Zimmermann et al., 2011). However, as yet these factors have not been linked to the differential restriction of IAV replication in epithelial cells compared with MΦ and DC. It is interesting to note that the NLRP3 transcript was only detected in mouse airway MΦ and not epithelial cells in an in vivo infection model (Allen et al., 2009), suggesting that MΦ and epithelial cells may differ in their capacity to sense viral RNA via the NLRP3 inflammasome.

Tetherin (also named BST-2) is another recently identified host antiviral restriction factor. Tetherin expression at the plasma membrane is induced by IFN and can block the release of enveloped viruses, including Ebola (Evans et al., 2010) and HIV-1 (Neil et al., 2008). HIV-1-encoded viral protein U (VPU) is able to counteract tetherin to promote viral release. Interestingly, HIV-1 expressing a mutant VPU with an impaired ability to antagonize tetherin did not replicate in MΦ (expressing high levels of tetherin), but was still able to replicate in peripheral blood leucocytes and to deplete CD4+ T-cells (both expressing comparatively low levels of tetherin) (Schindler et al., 2010). This implies that the ability of HIV-1-encoded VPU to antagonize tetherin may vary between cell types with differing tetherin

### Table 2. Infection of human DC with IAV

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<th>Able to infect immature DCs?</th>
<th>Productive in immature DCs?</th>
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<tr>
<td>Seasonal IAV</td>
<td>✓Bender et al. (1998);</td>
<td>XBender et al. (1998)</td>
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ND, Not done.

### Table 2. Infection of human DC with IAV

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ND, Not done.

Fig. 2. Functional consequences of H5N1 IAV infection of DC. (a) Following IAV infection of DC in the lung, infected DC traffic to the draining lymph node via the afferent lymphatic. (b) Upon entering the draining lymph node, infected DC may release infectious virus that could subsequently disseminate systemically via the efferent lymphatic or infect surrounding lymphocytes. Lymphocyte infection may lead to lymphocyte apoptosis and the leukopenia associated with H5N1 infections. It is also possible that DC, via cell surface DC-SIGN, may directly transfer infectious virus to other susceptible cells in the lymph node or tissues (Wang et al., 2008).
expression. Tetherin has also been proposed to modulate the budding of IAV virus-like particles (Watanabe et al., 2011; Yondola et al., 2011) and IAV and tetherin have been shown to co-localize at the plasma membrane of infected epithelial cell lines (Bruce et al., 2012). However, over-expression of tetherin by mammalian cell lines did not restrict release of infectious IAV (Bruce et al., 2012; Watanabe et al., 2011); although its role in modulating IAV release from infected MΦ or DC has yet to be reported. Together, these data demonstrate the importance of further studies to identify and characterize host-encoded IAV restriction factors. This, in turn, may lead to the identification of novel mechanisms by which some IAV strains have evolved to infect particular MΦ and DC subsets productively. Identification of host and/or viral factors that determine abortive versus productive infection in MΦ and DC is an exciting prospect for understanding the virulence and pathogenicity of IAV.

Concluding remarks

Abortive infection of MΦ and DC serves as a ‘dead end’ for most seasonal and low pathogenic strains of IAV and therefore contributes to effective host defence. The ability of some highly pathogenic strains of IAV to overcome this block and infect MΦ and DC productively is likely to have important consequences with respect to viral amplification, dissemination, and therefore, pathogenicity and immunogenicity. Identification of host or virus-encoded restriction factors that determine abortive versus productive infection in MΦ and DC will be an important step towards understanding the pathogenicity of different IAV strains.

Acknowledgements

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