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Dendritic cells and influenza A virus infection

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Abbreviations: cDC, conventional dendritic cell; DC, dendritic cell; DT, diphtheria toxin; iBALT, inducible bronchus-associated lymphoid tissue; IAV, influenza A virus; IFNR, interferon receptor; MHC, major histocompatibility class; NS1, nonstructural protein 1; pDC, plasmacytoid dendritic cell; IFN, interferon; SA, sialic acid; ssRNA, single-stranded RNA; TLR, Toll-like receptor

Influenza A virus (IAV) is a dangerous virus equipped with the potential to evoke widespread pandemic disease. The 2009 H1N1 pandemic highlights the urgency for developing effective therapeutics against IAV infection. Vaccination is a major weapon to combat IAV and efforts to improve current regimes are critically important. Here, we will review the role of dendritic cells (DCs), a pivotal cell type in the initiation of robust IAV immunity. The complexity of DC subset heterogeneity in the respiratory tract and lymph node that drains the IAV infected lung will be discussed, together with the varied and in some cases, conflicting contributions of individual DC populations to presenting IAV associated antigen to T cells.

Influenza A virus (IAV) is a negative sense, single-stranded RNA (ssRNA) virus that establishes an acute infection in the respiratory tract. Responsible for annual illness, hospitalizations and deaths, IAV is a dangerous virus that is also equipped with the potential to evoke widespread pandemic disease. The 2009 H1N1 pandemic highlights the urgency for developing effective therapeutics against IAV infection. Vaccination is a major weapon to combat IAV and efforts to improve current regimes are critically important. In order to do this, the mechanisms that underlie immunity to IAV need to be studied in detail.[1] Here, we will review the complex role of dendritic cells (DCs), a pivotal cell type in the initiation of robust IAV immunity (**Fig. 1**).

Dendritic Cell Heterogeneity

DCs are equipped with the capacity to display peptides derived from viral-associated antigen in the context of major histocompatibility class I (MHCI) and MHCII molecules to elicit $CD8^+$ and $CD4^+$ T cell immunity, respectively. The source of the peptides differ: cytosolic for MHCI- and exogenous for MHCII-restricted peptides. Notably, distinct DC subpopulations possess the ability to load peptides derived from exogenous antigens onto MHC class I molecules. This process is termed "cross-presentation."[2,3] Cross-presentation pathway is considered critical for generating $CD8^+$ T cell immunity against pathogens, such as IAV, that do not primarily infect DCs. Therefore, DCs are a heterogeneous population of cells, with different subsets displaying specialized antigen presentation functions.

To date, the identity of the DCs involved and the exact nature of the mechanisms utilized to initiate IAV-specific T cell immunity remain controversial. There is considerable complexity in identifying subpopulations of the DC family. Developmental stages are still being defined, together with increasing numbers of lineage markers to define end-stage subpopulations. Having said this, significant progress has identified distinct DC family members that can now be readily defined. DCs are routinely subdivided into two major subsets that include plasmacytoid DCs (pDCs) and myeloid DCs, with the latter commonly referred to as conventional DCs (cDCs).[4] pDCs are a major source of the antiviral cytokine interferon-α (IFNα).[5] cDCs that are isolated from the lymph node that drains the lung represent a mixture of lymphoid resident DCs that do not traffic to peripheral tissues, and tissue-derived, "migratory" DCs. At least three lymphoid resident DC subsets are described and can be subcategorized based on their expression of the lymphocyte markers CD4 and CD8 ($CD8^+$ DCs, $CD4^+$ DCs and $CD8^-CD4^-$ DCs).[6] Two migratory DC subsets are defined and are subdivided based on their expression of the mucosal α_E integrin marker CD103 and myeloid marker CD11b ($CD103^+CD11b^-$ and $CD103^-CD11b^+$).[7] In addition to cDCs and pDCs that are present in uninfected airways and lymph nodes, the inflammatory environment elicited by IAV infection recruits monocyte-derived "inflammatory DCs" to the lung parenchyma. These DCs are also referred to as tumor necrosis factor producing inducible nitric oxide synthetase-producing (TIP) DCs[8] or interferon killer (IKDCs).[9] Inflammatory DCs typically express CD11b and can be distinguished from conventional CD11b DCs by several specific lineage markers including Ly6C.[10] Finally, alveolar macrophages are a dominant cell type in the pulmonary tract that are often confused with DCs, given their expression of CD11c, but can be excluded from DC populations based on their high autofluorescence[11] as well as their exclusive expression of siglec F and CD2.[12] Therefore, many DC populations contribute to eliciting immunity to IAV both in the infected lung tissue and in the associated lymph node (**Table 1**).

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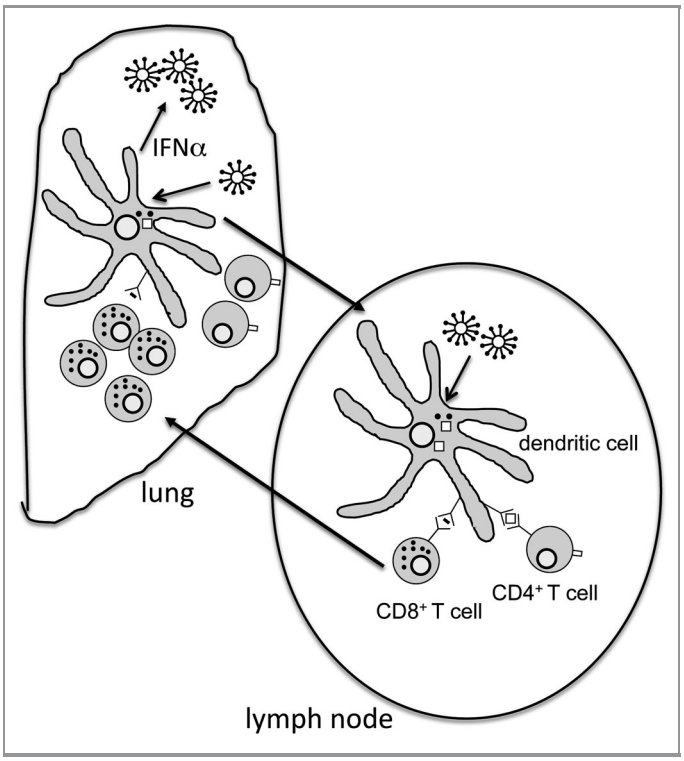

Figure 1. Dendritic cells (DCs) have multiple roles in immunity to influenza A virus (IAV) infection. DCs in the lung secrete pro-inflammatory cytokines including interferon-α (IFNα) following detection of IAV. DCs may become infected with IAV and/or traffic IAV antigen to the lymph node draining the lung. In the lymph node, DCs present IAV antigen to $CD4^+$ and $CD8^+$ T cells. IAV primed T cells migrate into the infected respiratory tract where they can again interact with lung resident DCs before differentiating into effector T cells that clear the IAV infection.

Detection of Influenza A Virus

DCs provide a first line of defense following IAV infection. Equipped with sensors to detect viral products, DCs alert the immune system to the presence of infectious virus. Invading IAV is detected by "pattern recognition receptors" (PRR). In early studies, the molecular signature generated by IAV was considered to be double-stranded viral RNA (dsRNA) recognized by Toll-like receptor 3 (TLR3).[13,14] A role for TLR3 was subsequently considered unlikely, however, given that the concentration of dsDNA generated by IAV is unlikely to be sufficient to signal TLR3.[15] Instead, the IAV polymerase generates uncapped ssRNA that serves as a unique molecular signature, readily identified by the immune system as foreign.[16] Interestingly, a growing number of cytosolic receptors that are capable of detecting viral products are being defined. These include members of the RNA helicase RIG-I-like receptor,[17] Nod-like receptor[18] and AIM2-like receptor[19] families. It is becoming increasingly apparent that multiple receptors are involved in detecting IAV. RIG-1,[16,20] but not MDA5,[16,21] detects IAV, which can occur independently of viral replication.[20] Members of the DExDc helicase family facilitate IAV recognition within myeloid DCs. These include DDX1-DDX21-DHX36[22] and DHX9.[23] IAV also triggers the NLRP3 inflammasome.[24-26] On the one hand, the NLRP3 inflammasome response to IAV was deemed critical for the development of adaptive immunity to IAV,[26] whereas others reported it as dispensable for adaptive IAV immunity, but critical for immediate innate immunity and tissue healing.[24,25] Notably, the NLRP3 inflammasome is not triggered by viral RNA, but by the ionic channel activity of IAV-encoded protein M2 that disturbs the intracellular ionic concentration.[27] In addition to cytosolic detectors, TLR7 is also implicated in IAV detection. Expressed in the endosomal compartments of plasmacytoid DCs and B cells, TLR7 detects IAV ssRNA.[28,29] Therefore, an array of molecules ensures that IAV is detected in the infected host.

Once IAV is detected, the individual PRR initiate multiple signaling cascades that aim to elicit innate immunity and facilitate viral eradication. In an attempt to evade such detection, IAV encodes non-structural protein 1 (NS1). Type 1 IFN induction is antagonized by NS1-mediated suppression of IFN-induced proteins including dsRNA-activated protein kinase, 2'-5-oligo (A) synthetase,[30-32] the transcription factors NFκB[33] and the IFN regulatory factor-3.[34] Containing an RNA-binding domain at its N terminus,[33] it was previously considered that NS1 sequestered

Table 1. Summary of dendritic cell subsets contributing to influenza A virus immunity

Population	Marker							
	CD11c	MHCII	CD45RA	sirpα	CD11b	CD103	Ly6C	CD8
Lymphoid resident DC								
plasmacytoid	int	+	+	–	–	–	–	+/–
$CD8^+$	hi	+	–	–	–	+/–	–	+
$CD8^–$	hi	+	–	+	+	–	–	–
Migratory DC								
CD11b	hi	+	–	+	+	–	–	–
CD103	hi	+	–	–	–	+	–	–
Inflammatory DC	hi	+	–	–	+	–	+	–

influenza A virus dsRNA.[35] Instead, NS1 forms a complex with RIG-1.[16] Finally, once IAV is cleared from the host, the inflammatory cascade must be shut down. Turning off the inflammatory response to IAV requires NLRX1, which acts to dampen RIG-1-mediated responses to IAV.[36] In summary, detection of IAV by DCs (and other cell types) is a complex process with the participation of several innate immune pathways and an active counterattack by the virus itself.

Initiation of T Cell Immunity to Influenza A Virus

While it is well-documented that $CD4^+$ and $CD8^+$ T cell immunity is initiated in response to IAV-associated antigens, the identification of the specific DC subsets that are responsible for presenting antigen to the respective T cell populations is the subject of intense ongoing research. Analysis of individual DC subsets and their role in antigen presentation following IAV infection has mostly relied on ex vivo analysis of isolated DC populations. In particular, the DC responsible for MHCI presentation of IAV antigen to $CD8^+$ T cells is a source of great debate. There are several conflicting reports as to which DC is responsible. On the one hand, MHCI antigen presentation of IAV antigen was attributed to $CD11b^+$ DCs,[37] while in a separate study, $CD103^+$ DCs were deemed the responsible subset.[38] The divergent results could not be explained by different methodologies used to detect IAV-derived antigen in the context of H-$2K^b$, as both studies utilized the same TCR-like mAb. Additional studies add to the confusion where IAV antigen is presented by MHCI expressed by both $CD103^+$ and $CD11b^+$ migratory DC subsets.[39,40] In one case, the $CD103^+$ DC subset preferentially drives naïve $CD8^+$ T cell differentiation,[39] while in the second study, this is achieved by $CD11b^+$ DCs.[40] The lymphoid resident $CD8^+$ DCs is also implicated in IAV MHCI antigen presentation to naïve $CD8^+$ T cells.[41-43] Disparities are also reported for the generation of memory $CD8^+$ T cells, with one report excluding migratory DC involvement[44] while another implicates antigen presentation by migratory $CD103^+$ DCs.[45] Therefore, currently there is no consensus as to which pulmonary DC subset is responsible for MHCI antigen presentation to $CD8^+$ T cells, either naïve or memory, following IAV infection. Identifying this DC subset is important as this cell type will be critical for vaccine strategies that aim to elicit $CD8^+$ cytotoxic T lymphocytes. For MHCII IAV antigen presentation, the data are less extensive, but again there is controversy as to which DC subset is responsible. In one study, $CD103^+$ DCs exclusively present IAV antigen via MHCII,[42] while in another study, both $CD103^+$ and $CD11b^+$ are implicated.[39]

Potentially, the difficulty in identifying the DC subset responsible for IAV antigen presentation reflects the difficulty in isolating DCs as strictly purified subsets. Therefore, in an attempt to resolve this debate, several studies have incorporated in vivo analysis. These studies have mostly focused on MHCI IAV antigen presentation and remain to be exploited for a more detailed evaluation of MHCII IAV antigen presentation. Intratracheal administration of diphtheria toxin (DT) transiently depletes lung $CD11c^+$ DCs in CD11c.DTR mice.[46] In this setting, $CD8^+$ T cell responses are impaired, implicating a requirement for migratory DCs in IAV MHCI antigen presentation.[39,42] The DT system must be viewed with caution; however, as other key populations including alveolar macrophages, which can harbor infectious IAV virions can also be eliminated. DT treatment may therefore remove a potential antigen source for DCs, rather than remove critical antigen presenting cells themselves. In addition, DT treatment has the potential to impact and reduce lymphoid resident DCs. Therefore, this system needs to be interpreted with caution. Another model relies on the langerin.DTR mice,[47] where DT administration specifically ablates langerin-expressing $CD103^+$ DCs. Treatment of langerin.DTR mice with DT following IAV infection results in reduced IAV MHCI antigen presentation and impaired anti-IAV $CD8^+$ T cell immunity.[42] This implicates an important role for $CD103^+$ DCs, although the fact that some anti-IAV $CD8^+$ T cell effectors are primed indicates that more than one DC subset can participate in the response. Again, however, this model has its caveats. Specifically, $CD103^+$ DC can be directly infected with IAV[43] and therefore can deliver IAV antigens to other DC subsets, including the lymphoid resident DCs.[41] Therefore, the loss of this population in DT-treated langerin.DTR mice could be misinterpreted as a key role in priming, rather than the provision of IAV antigen. This complexity is also applicable to the studies performed in mice lacking the chemokine receptor CCR7.[48] In the absence of CCR7, lung resident $CD11b^+$ and $CD103^+$ DCs are unable to emigrate from the lung to the draining lymph node.[39,49] Again, the role of $CD103^+$ DCs as an antigen source, rather than the DC subset that initiates MHCI antigen presentation must be considered. In addition, $CCR7^{-/-}$ lymphoid resident DCs may be unable to migrate to the paracortex to efficiently interact with naïve $CD8^+$ T cells. This again makes the contribution of lymphoid resident DCs difficult to exclude. Fortunately, there seems to be a consensus with regards to which DC populations are not involved in MHCI antigen presentation of IAV. pDCs do not present IAV antigens via MHCI to $CD8^+$ T cells,[39,41,42] although they are implicated in promoting anti-IAV B cell immunity.[42] $CD8^-$ lymph node resident DCs are not implicated in MHCI IAV antigen presentation.[37-43] Finally, inflammatory monocyte-derived DCs do not play a crucial role in the lung-draining lymph node with only very modest $CD8^+$ T cell priming elicited from these cells ex vivo.[39]

Lung Resident DCs and Influenza A Virus Infection

It is becoming increasingly evident that effector $CD8^+$ T cells continue to divide in the lung at sites of IAV pathology after their departure from the lymph nodes.[50] Such an event is attributed to DCs,[51] which are continually recruited to the lungs throughout the course of IAV infection.[10,51,52] These include pDCs and cDCs[39,40,42] as well as inflammatory monocyte-derived DCs[10,52] and IKDCs.[9]

The majority of the lung DC populations, with the exception of pDCs, appear to possess IAV antigens and can stimulate IAV-specific T cells ex vivo.[9,10,52] However, to date the overall

contribution and consequence of antigen presentation by these DC subsets remains unclear. Provision of local stimulation by respiratory tract DCs is required for optimal anti-IAV T cell immunity with this response being dependent on the provision of IL-15.[50] $CD8^+$ DCs, a subpopulation considered to reside only in the lymphoid compartment[4] is also reported to be present in the lungs following IAV infection.[51] It is unclear whether $CD8^+$ DCs precursors migrate from the blood to the infectious site, or whether the terminally differentiated cells migrate to the infected lung from the lymph node. In addition, it is unknown whether other lymphoid resident DC subsets, such as $CD4^+$ DCs, are also present in abundant numbers at the infectious site. One possibility is that $CD8^+$ DCs are associated with induced bronchus-associated lymphoid tissue (iBALT),[53] but this remains to be formally demonstrated. Maintenance of iBALT structures depends on the presence of lung DCs,[9] but the overall contribution of specific subsets within these structures and at other sites in the lung during influenza infection remains to be elucidated.

How Do Dendritic Cells Acquire Influenza A Virus Antigen?

As discussed, abundant evidence shows that respiratory tract DC traffic and present IAV-derived antigen to T cells in the lymph node that drains the IAV-infected lung.[9,42,43,54-57] In brief, there are two major mechanisms by which this antigen can be acquired. First, DCs may capture and phagocytose infected airway cells. This has been described for human immature DCs that phagocytose apoptotic IAV-infected monocytes in vitro.[58] In this case, the experiments were designed to exclude a role for the direct infection of DCs with the virus itself. IAV infected cells appear to undergo typical apoptosis with the display of phoshphatidylserine at the infected cell surface being the most likely trigger for phagocytosis.[59] CD36 and the $\alpha v\beta 5$ integrin are implicated as receptors that immature DCs employ to capture and acquire apoptotic IAV infected cell cargo.[60] Notably, excluding direct infection of DCs with IAV is not an easy undertaking. Utilizing an interesting and novel approach, Langlois et al. generated a virus containing hematopoietic-specific microRNA target sites inserted within the nucleoprotein gene. Infection with this virus results in undetectable transcription and replication in hematopoietic cells, including DCs, but intact IAV infection of epithelial cells. In this scenario, viral clearance and $CD8^+$ T cell responses are not altered. While it is difficult to rule out whether this approach is strictly excluding IAV infection in all relevant DC populations, this study does imply that DC acquisition of viral infected cells is a significant pathway by which DCs can elicit IAV immunity.[61]

The second major mechanism by which respiratory tract DCs acquire IAV antigen is to be directly infected with the virus itself. There are plenty of examples of IAV infection of DCs in vitro. Mouse bone marrow-derived DCs,[62-65] mouse splenic DCs,[66] human blood monocyte-derived DCs[67-70] and primary human myeloid, but not plasmacytoid, DCs[71] can be infected with IAV in vitro. Infecting DCs with IAV can result in expression of viral proteins,[66-70] but does not necessarily elicit infectious virions.[63] Whether we can extrapolate these findings to infection of pulmonary tract DCs during live IAV infection is debatable. DC subsets isolated directly from the pulmonary airways can also be infected with IAV in vitro, although this depends on the strain of virus[72,73] and the specific DC subset. $CD103^+$ DCs are the most susceptible to IAV infection, with $CD11b^+$ DCs displaying intermediate susceptibility and pDCs being the most resistant.[73,74] Isolation of DC subsets from IAV-infected mice confirms the differential infectivity of DC subsets, with the $CD103^+$ DCs being the major subset containing infectious IAV virus.[43,73] Differential infection of DC subsets correlates with their use of type I IFN receptor (IFNR) signaling, given that ablation of IFNR signaling enhances viral replication in $CD11b^+$ DCs.[43] Constraining IFN signaling in $CD103^+$ DCs to allow IAV infection may serve to promote IAV antigen presentation once $CD103^+$ DCs arrive in the lymph node. DCs isolated from the lungs of mice infected with highly pathogenic H1N1 and H5N1 IAV strains, were productively infected and could liberate infectious virus.[72]

Infection of DCs with IAV is primarily mediated by the recognition of cell surface sialic acid (SA) that is expressed by host cell glycoproteins and glycolipids. Binding of SA by viral hemagluttinin is the primary mode of IAV attachment; however, the presence of SA is not always sufficient for cell infection. Several C-type lectins known to be expressed by DCs are implicated in IAV entry including macrophage mannose receptor, a type I integral transmembrane protein with Ca^{2+}-dependent specificity for terminal D-mannose, N-acetyl-D-glucoamine and L-fructose; macrophage galactose type lectin,[75] a type II transmembrane glycoprotein with Ca^{2+}-dependent specificity for terminal galactose, Lewis-X structures and terminal GalNAc residues[75,76] and DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), a tetrameric type II transmembrane glycoprotein with Ca^{2+}-dependent lectin activity specific for high mannose.[77,78] Entry of IAV into DCs via these receptors may occur through direct endocytosis of bound IAV, or alternatively may involve the transfer of virus to additional co-receptors that facilitate its entry.[77] In an interesting study, Gonzalez et al. visualized the capture of inactivated IAV by spleen medullary $CD11c^+$ cells. In this case, SIGN-R1, another lectin with the ability to bind mannose-rich sugars was implicated.[79] This analysis was performed with inactivated virus in a vaccine setting and therefore the role of SIGN-R1 in live IAV infection remains to be elucidated.

IAV infection of DCs can induce DCs apoptosis.[64,70] As such, directly infecting DCs with IAV may be a viral mechanism to impair DC function and impede the initiation of an effective adaptive immune response. Indeed, IAV-infected human myeloid DCs are impaired in their ability to cross-present exogenous antigen via MHCI.[71] To overcome this, DCs directly infected with IAV may transfer their antigen to uninfected, functional DCs. This mechanism of IAV antigen presentation remains to be formally demonstrated but is likely to be a mode of antigen presentation by lymphoid resident DCs that participate in IAV T cell priming but do not access the site of infection themselves.

Conclusion

Ultimately, studies of IAV immunity will provide the foundation for strategies to combat IAV disease. DCs are critical participants in IAV detection and importantly, process and present IAV-associated antigen in a context that facilitates successful immunity. Here, we have summarized the complex role of DCs following IAV infection of the pulmonary tract. Specific DC subsets play critical role in both the infected lung itself and in the lymphoid organs that drain the respiratory tract. Several pathways discussed here are currently ongoing areas of intense and active research. In this case, we have attempted to discuss several studies that are often in complete disagreement, despite utilizing similar methodologies. Obviously, the complex network of DC subsets requires careful and elegant techniques for identifying and isolating purified DC populations from both the lymphoid organs and the infected pulmonary tissue. In vivo models of DC depletion are proving useful; however, they present several caveats that need to be carefully considered. All of the research discussed here has focused on studies undertaken with well-established mouse models of IAV. Moving forward, this knowledge must be considered in the context of human patients and human IAV disease.

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