

# Cell-surface receptors on macrophages and dendritic cells for attachment and entry of influenza virus

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## ABSTRACT

Airway M $\Phi$  and DCs are important components of innate host defense and can play a critical role in limiting the severity of influenza virus infection. Although it has been well established that cell-surface SA acts as a primary attachment receptor for IAV, the particular receptor(s) or coreceptor(s) that mediate IAV entry into any cell, including M $\Phi$  and DC, have not been clearly defined. Identifying which receptors are involved in attachment and entry of IAV into immune cells may have important implications in regard to understanding IAV tropism and pathogenesis. Recent evidence suggests that specialized receptors on M $\Phi$  and DCs, namely CLRs, can act as capture and/or entry receptors for many viral pathogens, including IAV. Herein, we review the early stages of infection of M $\Phi$  and DC by IAV. Specifically, we examine the potential role of CLRs expressed on M $\Phi$  and DC to act as attachment and/or entry receptors for IAV. *J. Leukoc. Biol.* 92: 97–106; 2012.

## Introduction

Influenza viruses are important respiratory pathogens belonging to the *Orthomyxoviridae* family of enveloped viruses and can be classified into three distinct types: A, B, and C, based on antigenically distinct, internal proteins. IAVs are the major etiological agent causing epidemics in humans and have the potential to cause pandemics. It is well established that cell-surface SA acts as the primary attachment receptor for IAV, following recognition by the viral HA. However, the process of

virus entry into cells, resulting in productive replication in airway epithelial cells or uptake by airway M $\Phi$  and DC, remains poorly defined. Specialized receptors expressed by M $\Phi$  and DC, including those of the CLR family, have emerged as important receptors for attachment and uptake of a range of viruses. We and others [1–4] have recently provided evidence that CLRs may also play a role in IAV infection of M $\Phi$  and DC.

## VIRAL DETERMINANTS OF IAV INFECTION: THE ENVELOPE GLYCOPROTEINS

The IAV genome is comprised of eight segments of single-stranded, negative-sense RNA, which encode at least 11 proteins. Two viral glycoproteins—the HA and the NA—protrude from the surface of the virion [5] and are key determinants in attachment, entry, and infection of target cells. The HA exists as a trimer of three identical monomers formed by noncovalent association. Each monomer consists of a globular head and a stalk region anchored in the viral envelope by a short hydrophobic sequence. Within the globular head is the receptor-binding site, a shallow pocket of highly conserved amino acids that interact with *N*-acetylneuraminic acid (commonly referred to as SA), expressed on oligosaccharide side-chains of cell-surface glycoproteins and glycolipids [6–8]. Following attachment, virus is internalized via endocytosis, and HA undergoes conformational changes in the low pH of the endosomal compartment to expose the fusion peptide at the N-terminus of the HA<sub>2</sub> subunit [9, 10]. Fusion of viral and endosomal membranes allows delivery of the viral nucleocapsid into the cell cytoplasm before entry into the nucleus to initiate viral replication [11].

NA is a tetrameric glycoprotein formed by the association of four identical monomers. Each monomer is composed of a globular head and a stalk region embedded in the viral envelope.

Abbreviations:  $\alpha(2,3)$ -Gal= $\alpha(2,3)$ -galactose, APC=antigen presenting cell, CLR=calcium-dependent (C-type) lectin receptor, CRD=carbohydrate recognition domain, DC-SIGN=DC-specific ICAM-3-grabbing nonintegrin, GalNAc=*N*-acetylgalactosamine, HCV=hepatitis C virus, IAV=influenza A virus, L-SIGN=DC-specific ICAM-3-grabbing nonintegrin liver-expressed homologue, M $\Phi$ =macrophage, MDCK=Madin-Darby canine kidney, MGL=macrophage galactose-type lectin, MMR=macrophage mannose receptor, NA=neuraminidase, PR8=A/PR/8/34, PRR=pattern recognition receptor, SA=sialic acid, SARS-CoV=severe acute respiratory syndrome-coronavirus, TLR=toll-like receptors, WNV=West Nile virus

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lope. The majority of antigenic sites is found on the globular head, as well the enzyme-active site that contains a number of charged amino acid residues [12]. The enzymatic function of NA is to cleave SA residues from the cell surface, enabling newly synthesized virions to detach from infected cells. In addition, NA cleaves SA from the IAV glycoproteins, thereby preventing HA-mediated self-aggregation of virus. Hence, HA and NA have opposing functions, such that high NA activity could result in inadequate attachment of HA to cell-surface SA, and conversely, excessive HA activity may limit release of newly formed virions from infected cells. A balance between the activities of the HA and NA is therefore critical in determining the efficiency of infection by different IAV [13, 14].

N-linked glycosylation is a common post-translational modification of mammalian glycoproteins, where oligosaccharide side-chains are attached through N-glycosidic linkages to the Asn residues of the Asn-X-Ser/Thr motif (where X may represent any amino acid except proline) [15]. HA and NA have potential sites for N-linked glycosylation, and the attached oligosaccharides are commonly a mixture of high-mannose (type I: branched structures terminating in the sugar mannose), complex (type II: branched structures terminating in sugars, such as mannose, galactose, GalNAc, and/or fucose), or hybrid-type oligosaccharides [16–19].

On HA, oligosaccharides, attached to the stalk region, are well-conserved between IAV strains and are important for HA stability and conformation [20–22], whereas glycosylation of the globular head can vary markedly in number, location, and type of oligosaccharide [23, 24]. HA serves as the major target for neutralizing antibodies, and glycans on the head of HA are likely to shield or modify antigenic sites [6]. Analysis of H1 sequences (1918–2010) indicate no glycans present on the top of the receptor-binding domain from 1918 and 2009 pandemic IAV strains [25], whereas the majority of seasonal H1 IAV strains is characterized by the presence of three to five glycosylation sites [26, 27]. Since their appearance in the human population in 1968, H3N2 viruses also acquired additional glycans on the head of HA [23, 28, 29], and recent strains carry as many as eight to 10 potential glycosylation sites [27]. These findings are consistent with a role for glycosylation in mediating evasion of antibody-mediated neutralization in the human population.

Loss or gain of glycans on HA can affect biological properties of IAV, such as changing the affinity of HA for host cell-surface receptors [30–34] or enhancing sensitivity to recognition by collectins of the innate immune system [34–38]. The mouse-adapted PR8 (H1N1) strain is notable for its lack of glycosylation on the head of HA [39]. We recently reported that addition of glycans to the PR8 HA or removal of glycosylation sites from the HA of seasonal strain A/Brazil/11/78 (H1N1) modulated sensitivity to collectins and virulence in a mouse model [40]. Therefore, the amount of glycosylation added to HA to circumvent humoral immune responses may be limited by the increased sensitivity of glycosylated IAV to components of innate defense.

## CELLULAR TARGETS OF IAV INFECTION

In humans, IAV infection is predominantly confined to the upper respiratory tract [41]. Thus, airway epithelial cells and antigen presenting cells (APCs) represent primary targets of IAV infection. It is well-established that attachment to and entry of IAV into epithelial cells result in productive infection, characterized by genomic replication, synthesis of viral proteins, assembly of virions, and release of infectious progeny. M $\Phi$  and DC from humans or mice are susceptible to IAV, yet the outcomes of infection are less clear. IAV infection of M $\Phi$ /DC has been associated with productive infection [42–44] but also with nonproductive infection, whereby genomic replication and synthesis of at least some viral proteins occur, yet the infectious cycle is blocked prior to virus release [2, 3, 45–50]. These differences may reflect the heterogeneous nature of M $\Phi$  and DC, particularly in the lung, as well as susceptibility of M $\Phi$ /DC to infection by the particular IAV strain(s) used.

## CELLULAR DETERMINANTS OF IAV INFECTION: CELL-SURFACE RECEPTORS

Virus attachment and entry into host cells are generally complex multistep processes involving sequential and/or simultaneous recognition of multiple cell-surface receptors. By definition, virus receptors are host-cell molecules (usually membrane-associated), which bind virus attachment proteins and are required for entry [51]. Coreceptors are cell-membrane proteins that bind specifically to viral proteins and are required for entry, in addition to the primary receptor (typically to ensure the continuation of the entry process after binding) [51].

### SA—the primary attachment receptor for IAV

SA is the primary attachment receptor for IAV, and there is an abundance of SA on the surface of mammalian cells [6]. In nature, SA is generally attached to the underlying galactose residues of glycans by  $\alpha(2,3)$ -Gal or  $\alpha(2,6)$ -Gal linkages [6]. The conformation of the SA linkage on host cells is an important determinant of virus tropism. In fact, residues within or in the vicinity of the receptor-binding pocket of the viral HA modulate which SA linkages are preferentially recognized [52–54]. In general, human IAV prefer SA linked in an  $\alpha(2,6)$ -Gal conformation, which is abundant in the human respiratory tract [52, 55–58], whereas avian IAV strains show a preference for  $\alpha(2,3)$ -Gal, which is expressed throughout the avian gastrointestinal tract [59–61]. Differences in receptor specificity between human and avian IAV are likely to be critical factors in limiting interspecies transmission, as well as modulating virulence.

Many studies have described the importance of SA in promoting IAV infection of epithelial and immune cells. Pretreatment of cells with bacterial sialidases removes cell-surface SA and renders cells resistant to IAV [2, 7, 62–64]. In addition, enzymatically swapping the linkage of SA expressed on the cell surface can alter susceptibility to infection by IAV strains with a particular receptor specificity [60, 64, 65].

## SA-independent entry of IAV

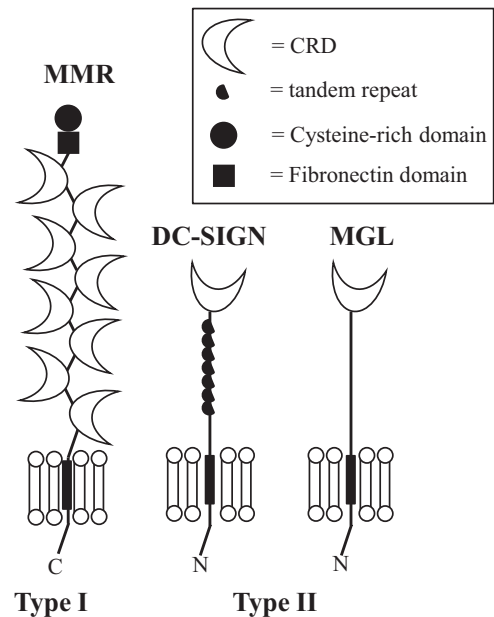
Although it is generally accepted that treatment with bacterial sialidase prevents IAV infection, desialylated mammalian MDCK cells can support infection, albeit at reduced levels [66], and desialylated human airway epithelial cells were permissive to IAV entry and at least the early stages of infection [57]. One study attempting to elucidate the nature of IAV receptors reported that a mutant CHO cell line deficient in *N*-linked glycans (Lec1 cells) was largely resistant to IAV infection, despite retaining full capacity for virus binding [67]. This led the authors to propose that SA expressed on glycolipids alone were insufficient for infection of CHO cells and that *N*-linked glycoprotein(s) were critical for infectious entry. Other studies have confirmed that binding of IAV to sialylated cell-surface receptors does not always result in receptor-mediated internalization [68, 69]. Rappoport et al. [70] examined binding of oligosaccharide probes to MDCK and Vero epithelial cell lines and obtained results consistent with the presence of cell-surface galectins and/or mannose-binding lectins, which they proposed could potentially recognize IAV and contribute to infection. Collectively, these findings imply that the presence of cell-surface SA is not always sufficient for IAV infection. Moreover, attachment and entry of IAV into cells can occur independently of SA. As proposed by Stray et al. [66], SA may enhance binding to the cell surface to increase subsequent and/or simultaneous interaction with secondary and/or coreceptors that are required for virus entry.

## C-TYPE LECTINS ON MΦ AND DC AS RECEPTORS FOR IAV

MΦ and DCs are immune cells equipped with an array of specialized pattern recognition receptors (PRRs), including scavenger receptors, toll-like receptors (TLRs), and CLR, to facilitate recognition and response to a range of microbial pathogens, including viruses [71–76]. Although the specific molecules mediating IAV entry into MΦ and DC have not been defined, we and others [1–4] have reported interactions between IAV and mannose-specific CLR (e.g., MMR and DC-SIGN/L-SIGN) and galactose-specific CLR (e.g., MGL).

### MMR

The MMR (CD206) is a type I integral transmembrane protein, 175–180 kDa in size, with an *N*-terminal cysteine-rich domain, a fibronectin type II repeat domain, an extracellular domain containing a tandem array of eight C-type lectin domains (also known as CRDs), a transmembrane domain, and a short cytoplasmic tail (Fig. 1). MMR exhibits  $\text{Ca}^{2+}$ -dependent specificity for terminal *D*-mannose, *N*-acetyl-*D*-glucosamine, and *L*-fucose [77]. The cytoplasmic tail contains two internalization motifs consistent with a role for MMR as a recycling receptor in the endocytic compartment [78–82]. MMR has been detected on human alveolar MΦ [83], mouse peritoneal MΦ [84], and rat splenic MΦ [85], as well as monocyte-derived human DCs [79, 86] and subsets of endothelial cells [87–89]. A wide range of bacteria, fungi, and protozoa is recognized by the MMR, including but not limited to *Mycobacterium tuberculosis*



**Figure 1. Schematic of CLR present on MΦ and DC that have been implicated as putative receptors for IAV.** MMR is a type I transmembrane protein containing multiple CRDs on a single polypeptide chain, as well as a cysteine-rich domain and a fibronectin domain. MGL and DC-SIGN are type II transmembrane proteins containing a single CRD, which clusters on the cell surface, forming homo-oligomers to increase binding avidity. C = carboxy terminus; N = amino terminus.

*sis*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Candida albicans*, and *Leishmania* spp. [72, 74].

The role of MMR as a viral receptor is summarized (see Table 1). MMR has been reported to enhance dengue virus infection of MΦ via recognition of oligosaccharides expressed on the viral envelope glycoprotein [90]. MMR also binds the gp120 envelope glycoprotein of HIV-1 [91] and promotes non-productive infection of MΦ [92]. For dengue virus and HIV-1, it is currently unclear if enhanced infection results from direct MMR-mediated endocytosis or by MMR-mediated attachment, promoting binding to additional receptor(s), which mediate virus entry. Aside from pathogen recognition, MMR also mediates antigen uptake and presentation to T lymphocytes [78, 86, 93–95] and has been implicated in lymphocyte homing and adhesion to lymphatic endothelium [96].

### MGL

MGL (CD301) is a 42-kDa type II transmembrane glycoprotein with a single CRD (Fig. 1), which forms homo-oligomers on the cell surface. It is a known endocytic receptor with internalization motifs located within the cytoplasmic tail [97–101]. In mice, two distinct isoforms of MGL, namely MGL-1 and MGL-2, have been described, which share 91.5% amino acid homology and have similar expression patterns [102]. However, MGL-1 displays  $\text{Ca}^{2+}$ -dependent specificity for terminal galactose, Lewis-X structures, and terminal GalNAc residues, whereas MGL-2 binds exclusively to terminal GalNAc residues [103, 104]. Like murine MGL-2, rat and human or-

thologs also have oligosaccharide specificity restricted to GalNAc residues [104]. In general, MGL is expressed by subsets of M $\Phi$  and monocyte-derived, immature DC (reviewed in ref. [104]). In humans, monocyte-derived DCs express moderate MGL levels, which decrease following maturation, although MGL is up-regulated on tolerogenic DCs generated during chronic inflammatory conditions and in the presence of steroids [105, 106], consistent with a role for MGL in immune regulation.

MMR interacts with many microbes, including enveloped and nonenveloped viruses; however, fewer studies have investigated the role of MGL as a PRR. To date, MGL has been reported to bind to *Schistosoma mansoni* and *Campylobacter jejuni* [107, 108]. MGL also recognizes the highly glycosylated, mucin-like domain in the viral envelope glycoprotein of certain filoviruses, such as Marburg and Ebola viruses (see Table 1) [109]. Transfection-based approaches have shown that MGL expression led to enhanced Marburg/Ebola virus infection, but whether this enhancement was mediated by direct MGL-mediated endocytosis or by promoting interaction with other entry receptors is unclear [109–111].

## DC-SIGN

DC-SIGN (CD209) is a tetrameric type II transmembrane glycoprotein (Fig. 1) with Ca<sup>2+</sup>-dependent lectin activity specific for high-mannose [112, 113] and fucosylated oligosaccharides [114]. It is an endocytic receptor with putative internalization domains located within the intracellular N-terminal domain [115]. DC-SIGN is expressed at high levels by monocyte- and CD34<sup>+</sup>-derived subsets of immature and mature DCs [116, 117], as well as alveolar M $\Phi$  [118]. DC-SIGN has a highly related homolog known as L-SIGN (DC-SIGNR), which shares 77% amino acid identity and Ca<sup>2+</sup>-dependent lectin specificity for high-mannose oligosaccharides. However, L-SIGN has a vastly different expression pattern and tissue localization (predominantly endothelial cells) and is not expressed by M $\Phi$  or DC (reviewed in ref. [119]). DC-SIGN is well-characterized as a cell-adhesion and pathogen receptor and promotes uptake of bacteria, fungi, and parasites, including *M. tuberculosis*, *Helicobacter pylori*, and *Leishmania* spp. (reviewed in refs. [72, 119]). Binding of DC-SIGN by distinct pathogens can lead to inhibition or promotion of particular T cell responses [120, 121], which may relate to the distinct signaling pathways induced by mannose- or fucose-rich ligands [122].

The role of DC-SIGN as a viral receptor is summarized (see Table 1). DC-SIGN has been implicated as an attachment receptor, resulting in enhanced infection of target cells by a range of viruses, including dengue virus [123], WNV [124], Ebola virus [125–127], and SARS-CoV [128]. However, the ability of DC-SIGN to act as a direct entry receptor or to enhance infection via interaction with alternative coreceptors is not clearly defined for many of these viruses (see Table 1).

## CLR-MEDIATED ENHANCEMENT OF IAV INFECTION OF M $\Phi$ AND DC

### IAV and MMR

Current evidence suggests that lectin-mediated interactions between IAV and MMR play a critical role in the infection of

M $\Phi$ . First, CRDs of the MMR have been shown to recognize N-linked glycans on the HA/NA of IAV in a Ca<sup>2+</sup>-dependent manner [2]. In addition, IAV infection of primary mouse M $\Phi$  but not epithelial cells was inhibited by mannan, a complex polymer of mannose residues [2, 3], and susceptibility of the J774 M $\Phi$  cell line to IAV infection correlated with levels of MMR expression [2]. Recently, we used direct-binding techniques to further characterize interactions between MMR and IAV. SA-dependent binding of IAV HA to MMR was reported, as well as SA-independent recognition of glycans on viral HA/NA by the lectin activity of MMR [3]. However, sialidase treatment of M $\Phi$  greatly reduced susceptibility to IAV infection, demonstrating that efficient infection requires contributions from SA and the lectin activity of MMR.

### IAV and MGL

In our efforts to characterize IAV-MMR interactions in more detail, virus-binding assays also revealed Ca<sup>2+</sup>-dependent binding of murine MGL to IAV [3]. Although MGL is sialylated, binding of MGL to IAV was blocked completely in the presence of galactose, indicating that SA expressed by MGL was not recognized by HA (or at least not by the HA of IAV strains used in the study). Moreover, IAV infection of M $\Phi$  was blocked by addition of asialofetuin, a multivalent ligand of MGL. These studies were not designed to discriminate between binding to MGL-1 or MGL-2; however, the M $\Phi$  cell lines used expressed only MGL-1, pointing to a role for this receptor in IAV infection of murine M $\Phi$ . As for MMR, treatment of MGL<sup>+</sup> M $\Phi$  with bacterial sialidase led to a marked reduction in susceptibility to IAV infection [3].

### IAV and DC-SIGN

Our studies examining murine MMR and MGL as IAV receptors were informative but relied on (i) correlation between receptor levels and susceptibility to infection and (ii) ability of receptor ligands to block IAV infection. For many viruses, identification of cell-surface receptors has been demonstrated following transfection of gene(s) encoding putative receptor(s) into cell lines that are resistant to infection, such that cells are rendered susceptible to virus entry. When studying IAV, such approaches are complicated by the abundant cell-surface SA on mammalian cells, and it has been difficult to find cell lines that are resistant to infection. Recently, we demonstrated that Lec2 cells, a mutant CHO cell line deficient in cell-surface SA [129, 130], bound IAV poorly and were largely resistant to IAV infection [1]. These studies defined an experimental system, in which SA-independent interactions between IAV and putative cell-surface receptors could be investigated. Expression of DC-SIGN (or its homologue L-SIGN) by SA-deficient Lec2 cells resulted in Ca<sup>2+</sup>-dependent IAV attachment and enhanced susceptibility to infection [1]. As infection was blocked by mannan, but not by pretreatment with bacterial sialidases, we concluded that DC-SIGN mediated recognition of mannose-rich glycans on IAV to promote SA-independent IAV infection. Wang et al. [4] also used a transfection-based approach to report that DC-SIGN can act as a receptor for H5N1 IAV. In this model, DC-SIGN-mediated H5N1 infection



of transfected cells was dependent on the presence of cell-surface SA (for infection in cis), and captured virus particles were also transferred to other permissive cells (for infection in trans).

## PROPOSED MODELS FOR CLR-MEDIATED ENHANCEMENT OF IAV INFECTION

As described above, MMR, MGL, and DC-SIGN can bind IAV and enhance IAV infection, yet the specific mechanisms by which they do this are not clear. A model for CLR-mediated IAV infection of SA-deficient Lec2 cells and MΦ/DC is depicted in Fig. 2.

### Lectin-mediated interactions among IAV and MMR, MGL, and DC-SIGN

Lectin-mediated binding of MMR, MGL, and DC-SIGN to glycans, expressed on the HA/NA glycoproteins of IAV, can occur independently of SA. Highly glycosylated strains of IAV bind these CLRs in a Ca<sup>2+</sup>-dependent manner [1, 3], and CLR-mediated infection was blocked by multivalent ligands of each CLR in a manner that corresponds to their expression on target cells [1–3]. Poorly glycosylated IAV, such as PR8, did not bind CLRs efficiently and were poor in their ability to infect CLR<sup>+</sup> cells [1, 3]. The contribution of specific glycans on the head of H1 [40] and H3 [38] IAV in determining sensitivity to soluble C-type lectins has been defined recently, and similar approaches will yield important information as to which glycans on different IAV subtypes are recognized by membrane-associated CLRs.

### SA-mediated interactions

Endogenous MMR, MGL, and DC-SIGN expressed on mammalian cells are sialylated, but each CLR retains lectin-binding activity for IAV in the absence of SA [1, 3]. Despite the ability of CLRs to bind IAV independently of SA, our studies using murine MΦ (which expressed MMR and/or MGL) suggest a dual dependence on SA expression and lectin-binding activity,

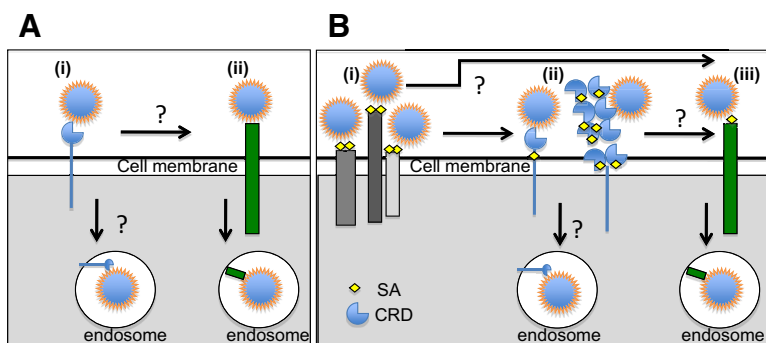
as desialylated MΦ were not susceptible to IAV infection [2]. Studies using DC-SIGN-transfected cell lines also showed that pretreatment with sialidase abrogated CLR-mediated enhancement of H5N1 IAV infection [4].

Although interactions between SA and IAV HA are of low affinity [131, 132], the abundance of SA on the surface of mammalian cells provides influenza virus with multiple receptors to increase binding avidity. Therefore, simultaneous binding of multiple HAs to SA would strengthen IAV binding to the cell surface and promote lectin-mediated binding of CLR. The ability of SA to concentrate virions at the cell surface might be particularly important on MΦ/DC, as CLRs are expressed at relatively low levels. For example, there are 10<sup>4</sup>–10<sup>5</sup> surface-binding sites for mannosylated ligand/cell for mouse and rabbit MΦ [133–135] and ~1 × 10<sup>3</sup> MGL molecules/cell for rat peritoneal MΦ [136]. Although detailed binding characteristics between IAV HA/NA and CLRs are yet to be determined, CLRs have been reported to bind other viral glycoproteins with high affinity [137].

For SA-independent infection of epithelial cells, Stray et al. [66] proposed that the requirement for initial interaction with SA to enrich IAV at the cell surface might be circumvented at higher virus concentrations. Similarly, the high levels of CLR expressed on SA-deficient Lec2-DC-SIGN cells [1] may also bypass the need for SA-mediated attachment and enrichment at the cell surface. Hence, although transfection-based approaches allow for isolation of putative IAV receptors in the absence of the confounding complexities of SA–HA interactions, it is critical to confirm the role of CLRs on relevant cell types (i.e., MΦ/DC), where SA is expressed. Such studies will allow us to determine the relevant contributions of SA and CLR to IAV infection of appropriate target cells.

### CLRs—attachment or entry receptors for IAV?

Our studies using MΦ/DC or CLR-transfected Lec2 cells do not discern between CLR-mediated endocytosis of IAV or whether CLRs represent an (additional) attachment receptor that passes IAV to other entry receptor(s) (Fig. 2A and B). For many viruses, it is unclear whether enhanced infection results



**Figure 2. Models for CLR-mediated enhancement of IAV infection.** (A) IAV infection of SA-deficient Lec2 CHO cells via CLR. (i) Lectin-mediated binding of the DC-SIGN CRD (shown in blue) to mannose-rich glycans on IAV HA/NA could lead to direct DC-SIGN-mediated endocytosis. Alternatively, (ii) IAV could be passed from DC-SIGN to additional cell-surface receptor(s) (the identity of which is currently unknown; shown in green), resulting in enhanced infection of Lec2 cells. Note that CLRs could also remain associated with IAV to facilitate entry via additional coreceptors. (B) IAV infection of MΦ and DC: a multistep process involving CLRs. (i) IAV HA binds to SA on cell-surface glycoproteins or glycolipids. Abundant cell-surface SA provides multiple sites for IAV attachment, thereby concentrating IAV at the cell surface. (ii)

Attachment to cell-surface SA facilitates lectin-mediated binding of CLRs to glycans on IAV HA/NA. Lectin-mediated binding may be strengthened by HA-mediated recognition of SA residues expressed on CLRs. Direct CLR-mediated entry may result, or (iii) CLRs may pass IAV to additional unidentified receptor(s) (shown in green) for virus entry. Binding of IAV to additional receptors could be SA-dependent or -independent. Interactions between IAV and unidentified receptors could also occur independently of CLRs [i.e., Step (i) followed by Step (iii)]. In addition, IAV could be endocytosed directly after binding to sialylated receptors in Step (i).

TABLE 1. MMR, MGL, and DC-SIGN CLR as Receptors for Viruses

CLR	Virus	Attachment receptor	Enhancement of infection	Mechanism of enhanced infection:	
				Direct entry	Coreceptor required
MMR	Dengue	Yes [90] <sup>a,b</sup>	Yes [90] <sup>a,b</sup>	?	?
	HIV-1	Yes [138] <sup>b</sup>	Yes [92] <sup>a,b</sup>	?	?
		Yes [92] <sup>a,b</sup>			
IAV	Yes [2] <sup>b</sup>	Yes [2] <sup>b</sup>	?	?	
	Yes [3] <sup>b</sup>	Yes [3] <sup>b</sup>			
MGL	Ebola	Yes [109] <sup>a</sup>	Yes [109] <sup>a</sup>	?	?
		Yes [111] <sup>a</sup>	Yes [111] <sup>a</sup>		
	Marburg	Yes [110] <sup>a</sup>	Yes [110] <sup>a</sup>	?	?
Yes [109] <sup>a</sup>		Yes [109] <sup>a</sup>			
DC-SIGN	IAV	Yes [3] <sup>b</sup>	Yes [3] <sup>b</sup>	?	?
		Yes [139]	Yes [140] <sup>a,b</sup>	? [140] <sup>a,b</sup>	Yes [140] <sup>a</sup>
	Dengue	Yes [139]	Yes [141] <sup>b</sup>	? [123] <sup>a</sup>	? [123] <sup>a</sup>
			Yes [123] <sup>a,b</sup>		
	HIV	Yes [142, 143] <sup>a,b</sup>			
	SIV	Yes [144, 145] <sup>a</sup>			
	HCV	Yes [146] <sup>a</sup>			
		Yes [147] <sup>b</sup>			
		Yes [148] <sup>a</sup>			
	CMV	Yes [149] <sup>a</sup>	Yes [149] <sup>a</sup>	?	?
	WNV	Yes [125] <sup>a,b</sup>	Yes [125] <sup>a,b</sup>	? [150] <sup>a,b</sup>	Yes [150] <sup>a,b</sup>
		Yes [150] <sup>a,b</sup>	Yes [150] <sup>a,b</sup>		
	Ebola	Yes [124] <sup>a</sup>	Yes [124] <sup>a</sup>	? [124] <sup>a</sup>	Yes [124] <sup>a</sup>
		Yes [126] <sup>a</sup>	Yes [126] <sup>a</sup>		Yes [126] <sup>a</sup>
				Yes [127] <sup>a</sup>	
Marburg	Yes [128] <sup>a</sup>	Yes [128] <sup>a</sup>	?	?	
SARS-CoV	Yes [151] <sup>a</sup>	Yes [151] <sup>a</sup>	? [151] <sup>a</sup>	? [151] <sup>a</sup>	
	Yes [128] <sup>a</sup>	Yes [128] <sup>a</sup>		Yes [128] <sup>a</sup>	
IAV	Yes [1] <sup>a</sup>	Yes [1] <sup>a</sup>	?	?	
IAV H5N1	Yes [4] <sup>a,b</sup>	Yes [4] <sup>a,b</sup>	?	Yes [4] <sup>a,b</sup>	

<sup>a</sup>Studies performed with transfected cells. <sup>b</sup>Studies performed with primary cells or cell lines.

from CLR-mediated endocytosis or following subsequent interaction with other entry receptors (Table 1). WT and endocytosis-defective DC-SIGN permitted dengue virus infection of transfected cells, indicating that endocytosis via DC-SIGN itself was not essential for infection [140]. Similar approaches will be used to determine whether CLRs can act as direct entry receptors for IAV. As well as promoting infection in cis, CLRs on MΦ/DC can capture and sequester virus, which may then be passed to other permissive cells. In this way, DC-SIGN promotes in trans infection by H5N1 IAV [4], HIV-1 [142], HCV [146], CMV [149], and Ebola viruses [127]. MMR on MΦ has also been reported to facilitate in trans infection of T cells by HIV-1 [138]. These represent additional mechanisms by which CLR–virus interactions can modulate virus dissemination during infection.

**CONCLUDING REMARKS, IMPLICATIONS, AND SIGNIFICANCE**

Many viruses use a two-step infection process, whereby virus initially binds to an abundant receptor (e.g., SA or heparin sulfate), via a low-affinity interaction, to promote contact with additional receptor(s), which are required for virus entry. For IAV, it seems likely that multiple low-affinity interactions between the viral HA and SA concentrate virus at the cell sur-

face, allowing it to “browse” or “roam” the cell surface until it contacts secondary receptor(s), as posited by Burnet [152]. On MΦ and DC, lectin-mediated binding by CLRs may represent one pathway by which IAV entry and infection can occur. However, at present, it is not clear whether CLRs themselves act as endocytic receptors for IAV or if additional receptors and/or coreceptors are required for virus entry. Understanding the specific mechanisms by which MΦ and DC recognize and internalize IAV may provide important information relevant to the tropism of different IAV for particular airway cells and therefore, pathogenesis. For example, mouse-adapted IAVs, such as PR8, evade detection by CLR [2, 3], infect airway MΦ poorly [1, 2, 153], and induce severe disease in mice following intranasal infection [154]. Moreover, in mice α(2,6)-Gal SA is the predominant linkage of MΦ compared with α(2,3)-Gal SA on epithelial cells [64, 155, 156], suggesting that the particular linkage of SA may be an important factor in recruiting particular IAV to different airway cells.

**AUTHORSHIP**

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## REFERENCES

- Londrigan, S. L., Turville, S. G., Tate, M. D., Deng, Y. M., Brooks, A. G., Reading, P. C. (2011) N-linked glycosylation facilitates sialic acid-independent attachment and entry of influenza A viruses into cells expressing DC-SIGN or L-SIGN. *J. Virol.* **85**, 2990–3000.
- Reading, P. C., Miller, J. L., Anders, E. M. (2000) Involvement of the mannose receptor in infection of macrophages by influenza virus. *J. Virol.* **74**, 5190–5197.
- Upham, J. P., Pickett, D., Irimura, T., Anders, E. M., Reading, P. C. (2010) Macrophage receptors for influenza A viruses: role of the macrophage galactose-type lectin and mannose receptor in viral entry. *J. Virol.* **84**, 3730–3737.
- Wang, S. F., Huang, J. C., Lee, Y. M., Liu, S. J., Chan, Y. J., Chau, Y. P., Chong, P., Chen, Y. M. (2008) DC-SIGN mediates avian H5N1 influenza virus infection in cis and in trans. *Biochem. Biophys. Res. Commun.* **373**, 561–566.
- Compans, R. W., Klenk, H. D., Caligiuri, L. A., Choppin, P. W. (1970) Influenza virus proteins. I. Analysis of polypeptides of the virion and identification of spike glycoproteins. *Virology* **42**, 880–889.
- Skehel, J. J., Wiley, D. C. (2000) Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu. Rev. Biochem.* **69**, 531–569.
- Wiley, D. C., Skehel, J. J. (1987) The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu. Rev. Biochem.* **56**, 365–394.
- Wilson, I. A., Cox, N. J. (1990) Structural basis of immune recognition of influenza virus hemagglutinin. *Annu. Rev. Immunol.* **8**, 737–771.
- Lai, A. L., Tamm, L. K. (2007) Locking the kink in the influenza hemagglutinin fusion domain structure. *J. Biol. Chem.* **282**, 23946–23956.
- Lin, Y. P., Wharton, S. A., Martin, J., Skehel, J. J., Wiley, D. C., Steinhauer, D. A. (1997) Adaptation of egg-grown and transfectant influenza viruses for growth in mammalian cells: selection of hemagglutinin mutants with elevated pH of membrane fusion. *Virology* **233**, 402–410.
- Cross, K. J., Burleigh, L. M., Steinhauer, D. A. (2001) Mechanisms of cell entry by influenza virus. *Expert Rev. Mol. Med.* **3**, 1–18.
- Gong, J., Xu, W., Zhang, J. (2007) Structure and functions of influenza virus neuraminidase. *Curr. Med. Chem.* **14**, 113–122.
- Mohsin, M. A., Morris, S. J., Smith, H., Sweet, C. (2002) Correlation between levels of apoptosis, levels of infection and haemagglutinin receptor binding interaction of various subtypes of influenza virus: does the viral neuraminidase have a role in these associations? *Virus Res.* **85**, 123–131.
- Wagner, R., Matrosovich, M., Klenk, H. D. (2002) Functional balance between haemagglutinin and neuraminidase in influenza virus infections. *Rev. Med. Virol.* **12**, 159–166.
- Larkin, A., Imperiali, B. (2011) The expanding horizons of asparagine-linked glycosylation. *Biochemistry* **50**, 4411–4426.
- Basak, S., Pritchard, D. G., Bhowan, A. S., Compans, R. W. (1981) Glycosylation sites of influenza viral glycoproteins: characterization of tryptic glycopeptides from the A/USSR(H1N1) hemagglutinin glycoprotein. *J. Virol.* **37**, 549–558.
- Collins, J. K., Knight, C. A. (1978) Purification of the influenza hemagglutinin glycoprotein and characterization of its carbohydrate components. *J. Virol.* **26**, 457–467.
- Reading, P. C., Tate, M. D., Pickett, D. L., Brooks, A. G. (2007) Glycosylation as a target for recognition of influenza viruses by the innate immune system. *Adv. Exp. Med. Biol.* **598**, 279–292.
- Ward, C. W., Dopheide, T. A. (1981) Amino acid sequence and oligosaccharide distribution of the hemagglutinin from an early Hong Kong influenza virus variant A/Aichi/2/68 (X-31). *Biochem. J.* **193**, 953–962.
- Daniels, R., Kurowski, B., Johnson, A. E., Hebert, D. N. (2003) N-linked glycans direct the cotranslational folding pathway of influenza hemagglutinin. *Mol. Cell* **11**, 79–90.
- Nobusawa, E., Aoyama, T., Kato, H., Suzuki, Y., Tateno, Y., Nakajima, K. (1991) Comparison of complete amino acid sequences and receptor-binding properties among 13 serotypes of hemagglutinins of influenza A viruses. *Virology* **182**, 475–485.
- Roberts, P. C., Garten, W., Klenk, H. D. (1993) Role of conserved glycosylation sites in maturation and transport of influenza A virus hemagglutinin. *J. Virol.* **67**, 3048–3060.
- Abe, Y., Takashita, E., Sugawara, K., Matsuzaki, Y., Muraki, Y., Hongo, S. (2004) Effect of the addition of oligosaccharides on the biological activities and antigenicity of influenza A/H3N2 virus hemagglutinin. *J. Virol.* **78**, 9605–9611.
- Wagner, R., Wolff, T., Herwig, A., Pleschka, S., Klenk, H. D. (2000) Interdependence of hemagglutinin glycosylation and neuraminidase as regulators of influenza virus growth: a study by reverse genetics. *J. Virol.* **74**, 6316–6323.
- Wei, C. J., Boyington, J. C., Dai, K., Houser, K. V., Pearce, M. B., Kong, W. P., Yang, Z. Y., Tumpey, T. M., Nabel, G. J. (2010) Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. *Sci. Transl. Med.* **2**, 24ra21.
- Igarashi, M., Ito, K., Kida, H., Takada, A. (2008) Genetically destined potentials for N-linked glycosylation of influenza virus hemagglutinin. *Virology* **376**, 323–329.
- Zhang, M., Gaschen, B., Blay, W., Foley, B., Haigwood, N., Kuiken, C., Korber, B. (2004) Tracking global patterns of N-linked glycosylation site variation in highly variable viral glycoproteins: HIV, SIV, and HCV envelopes and influenza hemagglutinin. *Glycobiology* **14**, 1229–1246.
- Cherry, J. L., Lipman, D. J., Nikolskaya, A., Wolf, Y. I. (2009) Evolutionary dynamics of N-glycosylation sites of influenza virus hemagglutinin. *PLoS Curr.* **1**, RRN1001.
- Seidel, W., Kunkel, F., Geisler, B., Garten, W., Herrmann, B., Dohner, L., Klenk, H. D. (1991) Intraepidemic variants of influenza virus H3 hemagglutinin differing in the number of carbohydrate side chains. *Arch. Virol.* **120**, 289–296.
- Anders, E. M., Hartley, C. A., Jackson, D. C. (1990) Bovine and mouse serum  $\beta$  inhibitors of influenza A viruses are mannose-binding lectins. *Proc. Natl. Acad. Sci. USA* **87**, 4485–4489.
- Gambaryan, A. S., Marinina, V. P., Tuzikov, A. B., Bovin, N. V., Rudneva, I. A., Sinityn, B. V., Shilov, A. A., Matrosovich, M. N. (1998) Effects of host-dependent glycosylation of hemagglutinin on receptor-binding properties on H1N1 human influenza A virus grown in MDCK cells and in embryonated eggs. *Virology* **247**, 170–177.
- Hartley, C. A., Jackson, D. C., Anders, E. M. (1992) Two distinct serum mannose-binding lectins function as  $\beta$  inhibitors of influenza virus: identification of bovine serum  $\beta$  inhibitor as conglutinin. *J. Virol.* **66**, 4358–4363.
- Ohuchi, M., Ohuchi, R., Feldmann, A., Klenk, H. D. (1997) Regulation of receptor binding affinity of influenza virus hemagglutinin by its carbohydrate moiety. *J. Virol.* **71**, 8377–8384.
- Tate, M. D., Job, E. R., Brooks, A. G., Reading, P. C. (2011) Glycosylation of the hemagglutinin modulates the sensitivity of H3N2 influenza viruses to innate proteins in airway secretions and virulence in mice. *Virology* **413**, 84–92.
- Hartshorn, K. L., Webby, R., White, M. R., Tecle, T., Pan, C., Boucher, S., Moreland, R. J., Crouch, E. C., Scheule, R. K. (2008) Role of viral hemagglutinin glycosylation in anti-influenza activities of recombinant surfactant protein D. *Respir. Res.* **9**, 65.
- Reading, P. C., Morey, L. S., Crouch, E. C., Anders, E. M. (1997) Collectin-mediated antiviral host defense of the lung: evidence from influenza virus infection of mice. *J. Virol.* **71**, 8204–8212.
- Reading, P. C., Pickett, D. L., Tate, M. D., Whitney, P. G., Job, E. R., Brooks, A. G. (2009) Loss of a single N-linked glycan from the hemagglutinin of influenza virus is associated with resistance to collectins and increased virulence in mice. *Respir. Res.* **10**, 117.
- Vigerust, D. J., Ulett, K. B., Boyd, K. L., Madsen, J., Hawgood, S., McCullers, J. A. (2007) N-linked glycosylation attenuates H3N2 influenza viruses. *J. Virol.* **81**, 8593–8600.
- Caton, A. J., Brownlee, G. G., Yewdell, J. W., Gerhard, W. (1982) The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell* **31**, 417–427.
- Tate, M. D., Brooks, A. G., Reading, P. C. (2011) Specific sites of N-linked glycosylation on the hemagglutinin of H1N1 subtype influenza A virus determine sensitivity to inhibitors of the innate immune system and virulence in mice. *J. Immunol.* **187**, 1884–1894.
- Taubenberger, J. K., Morens, D. M. (2008) The pathology of influenza virus infections. *Annu. Rev. Pathol.* **3**, 499–522.
- Cheung, C. Y., Poon, L. L., Lau, A. S., Luk, W., Lau, Y. L., Shortridge, K. F., Gordon, S., Guan, Y., Peiris, J. S. (2002) Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* **360**, 1831–1837.
- Perrone, L. A., Plowden, J. K., Garcia-Sastre, A., Katz, J. M., Tumpey, T. M. (2008) H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog.* **4**, e1000115.
- Yu, W. C., Chan, R. W., Wang, J., Travanty, E. A., Nicholls, J. M., Peiris, J. S., Mason, R. J., Chan, M. C. (2011) Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses. *J. Virol.* **85**, 6844–6855.



45. Bender, A., Albert, M., Reddy, A., Feldman, M., Sauter, B., Kaplan, G., Hellman, W., Bhardwaj, N. (1998) The distinctive features of influenza virus infection of dendritic cells. *Immunobiology* **198**, 552–567.
46. Hennet, T., Ziltener, H. J., Frei, K., Peterhans, E. (1992) A kinetic study of immune mediators in the lungs of mice infected with influenza A virus. *J. Immunol.* **149**, 932–939.
47. Osterlund, P., Pirhonen, J., Ikonen, N., Ronkko, E., Strengell, M., Makela, S. M., Broman, M., Hamming, O. J., Hartmann, R., Ziegler, T., Julkunen, I. (2010) Pandemic H1N1 2009 influenza A virus induces weak cytokine responses in human macrophages and dendritic cells and is highly sensitive to the antiviral actions of interferons. *J. Virol.* **84**, 1414–1422.
48. Rodgers, B., Mims, C. A. (1981) Interaction of influenza virus with mouse macrophages. *Infect. Immun.* **31**, 751–757.
49. Rodgers, B. C., Mims, C. A. (1982) Influenza virus replication in human alveolar macrophages. *J. Med. Virol.* **9**, 177–184.
50. Wells, M. A., Albrecht, P., Daniel, S., Ennis, F. A. (1978) Host defense mechanisms against influenza virus: interaction of influenza virus with murine macrophages in vitro. *Infect. Immun.* **22**, 758–762.
51. Dimitrov, D. S. (2004) Virus entry: molecular mechanisms and biomedical applications. *Nat. Rev. Microbiol.* **2**, 109–122.
52. Rogers, G. N., Paulson, J. C. (1983) Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology* **127**, 361–373.
53. Rogers, G. N., Paulson, J. C., Daniels, R. S., Skehel, J. J., Wilson, I. A., Wiley, D. C. (1983) Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* **304**, 76–78.
54. Russell, R. J., Stevens, D. J., Haire, L. F., Gamblin, S. J., Skehel, J. J. (2006) Avian and human receptor binding by hemagglutinins of influenza A viruses. *Glycoconj. J.* **23**, 85–92.
55. Connor, R. J., Kawaoka, Y., Webster, R. G., Paulson, J. C. (1994) Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* **205**, 17–23.
56. Rogers, G. N., D'Souza, B. L. (1989) Receptor binding properties of human and animal H1 influenza virus isolates. *Virology* **173**, 317–322.
57. Thompson, C. I., Barclay, W. S., Zambon, M. C., Pickles, R. J. (2006) Infection of human airway epithelium by human and avian strains of influenza A virus. *J. Virol.* **80**, 8060–8068.
58. Webby, R., Hoffmann, E., Webster, R. (2004) Molecular constraints to interspecies transmission of viral pathogens. *Nat. Med.* **10**, S77–S81.
59. Ito, T., Couceiro, J. N., Kelm, S., Baum, L. G., Krauss, S., Castrucci, M. R., Donatelli, I., Kida, H., Paulson, J. C., Webster, R. G., Kawaoka, Y. (1998) Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J. Virol.* **72**, 7367–7373.
60. Matrosovich, M. N., Matrosovich, T. Y., Gray, T., Roberts, N. A., Klenk, H. D. (2004) Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc. Natl. Acad. Sci. USA* **101**, 4620–4624.
61. Suzuki, Y. (2005) Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses. *Biol. Pharm. Bull.* **28**, 399–408.
62. Ochiai, H., Kurokawa, M., Hayashi, K., Niwayama, S. (1988) Antibody-mediated growth of influenza A NWS virus in macrophagelike cell line P388D1. *J. Virol.* **62**, 20–26.
63. Tamura, M., Webster, R. G., Ennis, F. A. (1991) Antibodies to HA and NA augment uptake of influenza A viruses into cells via Fc receptor entry. *Virology* **182**, 211–219.
64. Tate, M. D., Brooks, A. G., Reading, P. C. (2011) Correlation between sialic acid expression and infection of murine macrophages by different strains of influenza virus. *Microbes Infect.* **13**, 202–207.
65. Shinya, K., Ebina, M., Yamada, S., Ono, M., Kasai, N., Kawaoka, Y. (2006) Avian flu: influenza virus receptors in the human airway. *Nature* **440**, 435–436.
66. Stray, S. J., Cummings, R. D., Air, G. M. (2000) Influenza virus infection of desialylated cells. *Glycobiology* **10**, 649–658.
67. Chu, V. C., Whittaker, G. R. (2004) Influenza virus entry and infection require host cell N-linked glycoprotein. *Proc. Natl. Acad. Sci. USA* **101**, 18153–18158.
68. Carroll, S. M., Paulson, J. C. (1985) Differential infection of receptor-modified host cells by receptor-specific influenza viruses. *Virus Res.* **3**, 165–179.
69. Williams, S. P., Robertson, J. S. (1993) Analysis of the restriction to the growth of nonegg-adapted human influenza virus in eggs. *Virology* **196**, 660–665.
70. Rapoport, E. M., Mochalova, L. V., Gabius, H. J., Romanova, J., Bovin, N. V. (2006) Search for additional influenza virus to cell interactions. *Glycoconj. J.* **23**, 115–125.
71. Areschoug, T., Gordon, S. (2008) Pattern recognition receptors and their role in innate immunity: focus on microbial protein ligands. *Contrib. Microbiol.* **15**, 45–60.
72. Osorio, F., Reis e Sousa, C. (2011) Myeloid C-type lectin receptors in pathogen recognition and host defense. *Immunity* **34**, 651–664.
73. Pluddemann, A., Mukhopadhyay, S., Gordon, S. (2006) The interaction of macrophage receptors with bacterial ligands. *Expert Rev. Mol. Med.* **8**, 1–25.
74. Pluddemann, A., Mukhopadhyay, S., Gordon, S. (2011) Innate immunity to intracellular pathogens: macrophage receptors and responses to microbial entry. *Immunol. Rev.* **240**, 11–24.
75. Taylor, P. R., Martinez-Pomares, L., Stacey, M., Lin, H. H., Brown, G. D., Gordon, S. (2005) Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* **23**, 901–944.
76. Van Vliet, S. J., Garcia-Vallejo, J. J., van Kooyk, Y. (2008) Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses. *Immunol. Cell Biol.* **86**, 580–587.
77. Pontow, S. E., Kery, V., Stahl, P. D. (1992) Mannose receptor. *Int. Rev. Cytol.* **137B**, 221–244.
78. Engering, A. J., Cella, M., Fluitsma, D., Brockhaus, M., Hoefsmit, E. C., Lanzavecchia, A., Pieters, J. (1997) The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur. J. Immunol.* **27**, 2417–2425.
79. Engering, A. J., Cella, M., Fluitsma, D. M., Hoefsmit, E. C., Lanzavecchia, A., Pieters, J. (1997) Mannose receptor mediated antigen uptake and presentation in human dendritic cells. *Adv. Exp. Med. Biol.* **417**, 183–187.
80. Lennartz, M. R., Cole, F. S., Shepherd, V. L., Wileman, T. E., Stahl, P. D. (1987) Isolation and characterization of a mannose-specific endocytosis receptor from human placenta. *J. Biol. Chem.* **262**, 9942–9944.
81. Stahl, P., Schlesinger, P. H., Sigardson, E., Rodman, J. S., Lee, Y. C. (1980) Receptor-mediated pinocytosis of mannose glycoconjugates by macrophages: characterization and evidence for receptor recycling. *Cell* **19**, 207–215.
82. Tietze, C., Schlesinger, P., Stahl, P. (1982) Mannose-specific endocytosis receptor of alveolar macrophages: demonstration of two functionally distinct intracellular pools of receptor and their roles in receptor recycling. *J. Cell Biol.* **92**, 417–424.
83. Stephenson, J. D., Shepherd, V. L. (1987) Purification of the human alveolar macrophage mannose receptor. *Biochem. Biophys. Res. Commun.* **148**, 883–889.
84. Stahl, P., Gordon, S. (1982) Expression of a mannosyl-fucosyl receptor for endocytosis on cultured primary macrophages and their hybrids. *J. Cell Biol.* **93**, 49–56.
85. Harms, G., Dijkstra, C. D., Hardonk, M. J. (1990) Glycosyl receptors in macrophage subpopulations of rat spleen and lymph node. A comparative study using neoglycoproteins and monoclonal antibodies ED1, ED2 and ED3. *Cell Tissue Res.* **262**, 35–40.
86. Sallusto, F., Cella, M., Danieli, C., Lanzavecchia, A. (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* **182**, 389–400.
87. Linehan, S. A., Martinez-Pomares, L., Stahl, P. D., Gordon, S. (1999) Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: in situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. *J. Exp. Med.* **189**, 1961–1972.
88. Magnusson, S., Berg, T. (1993) Endocytosis of ricin by rat liver cells in vivo and in vitro is mainly mediated by mannose receptors on sinusoidal endothelial cells. *Biochem. J.* **291**, 749–755.
89. Shepherd, V. L., Tarnowski, B. I., McLaughlin, B. J. (1991) Isolation and characterization of a mannose receptor from human pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* **32**, 1779–1784.
90. Miller, J. L., de Wet, B. J., Martinez-Pomares, L., Radcliffe, C. M., Dwek, R. A., Rudd, P. M., Gordon, S. (2008) The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog.* **4**, e17.
91. Lai, J., Bernhard, O. K., Turville, S. G., Harman, A. N., Wilkinson, J., Cunningham, A. L. (2009) Oligomerization of the macrophage mannose receptor enhances gp120-mediated binding of HIV-1. *J. Biol. Chem.* **284**, 11027–11038.
92. Trujillo, J. R., Rogers, R., Molina, R. M., Dangond, F., McLane, M. F., Essex, M., Brain, J. D. (2007) Noninfectious entry of HIV-1 into peripheral and brain macrophages mediated by the mannose receptor. *Proc. Natl. Acad. Sci. USA* **104**, 5097–5102.
93. Prigozy, T. I., Sieling, P. A., Clemens, D., Stewart, P. L., Behar, S. M., Porcelli, S. A., Brenner, M. B., Modlin, R. L., Kronenberg, M. (1997) The mannose receptor delivers lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. *Immunity* **6**, 187–197.
94. Ramakrishna, V., Trembl, J. F., Vitale, L., Connolly, J. E., O'Neill, T., Smith, P. A., Jones, C. L., He, L. Z., Goldstein, J., Wallace, P. K., Keler, T., Endres, M. J. (2004) Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma T cell responses via multiple HLA molecules. *J. Immunol.* **172**, 2845–2852.
95. Wollenberg, A., Mommaas, M., Ooppel, T., Schottdorf, E. M., Gunther, S., Moderer, M. (2002) Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J. Invest. Dermatol.* **118**, 327–334.
96. Irjala, H., Alanen, K., Grenman, R., Heikkila, P., Joensuu, H., Jalkanen, S. (2003) Mannose receptor (MR) and common lymphatic endothelial and vascular endothelial receptor (CLEVER)-1 direct the binding of cancer cells to the lymph vessel endothelium. *Cancer Res.* **63**, 4671–4676.



97. Denda-Nagai, K., Kubota, N., Tsuiji, M., Kamata, M., Irimura, T. (2002) Macrophage C-type lectin on bone marrow-derived immature dendritic cells is involved in the internalization of glycosylated antigens. *Glycobiology* **12**, 443–450.
98. Higashi, N., Fujioka, K., Denda-Nagai, K., Hashimoto, S., Nagai, S., Sato, T., Fujita, Y., Morikawa, A., Tsuiji, M., Miyata-Takeuchi, M., Sano, Y., Suzuki, N., Yamamoto, K., Matsushima, K., Irimura, T. (2002) The macrophage C-type lectin specific for galactose/N-acetylgalactosamine is an endocytic receptor expressed on monocyte-derived immature dendritic cells. *J. Biol. Chem.* **277**, 20686–20693.
99. Ozaki, K., Itoh, N., Kawasaki, T. (1993) Role of tyrosine-5 in the cytoplasmic tail of the macrophage asialoglycoprotein receptor in the rapid internalization of ligands. *J. Biochem.* **113**, 271–276.
100. Valladeau, J., Duvert-Frances, V., Pin, J. J., Kleijmeer, M. J., Ait-Yahia, S., Ravel, O., Vincent, C., Vega Jr., F., Helms, A., Gorman, D., Zurawski, S. M., Zurawski, G., Ford, J., Saeland, S. (2001) Immature human dendritic cells express asialoglycoprotein receptor isoforms for efficient receptor-mediated endocytosis. *J. Immunol.* **167**, 5767–5774.
101. Van Vliet, S. J., Aarnoudse, C. A., Broks-van den Berg, V. C., Boks, M., Geijtenbeek, T. B., van Kooyk, Y. (2007) MGL-mediated internalization and antigen presentation by dendritic cells: a role for tyrosine-5. *Eur. J. Immunol.* **37**, 2075–2081.
102. Tsuiji, M., Fujimori, M., Ohashi, Y., Higashi, N., Onami, T. M., Hedrick, S. M., Irimura, T. (2002) Molecular cloning and characterization of a novel mouse macrophage C-type lectin, mMGL2, which has a distinct carbohydrate specificity from mMGL1. *J. Biol. Chem.* **277**, 28892–28901.
103. Singh, S. K., Streng-Ouweland, I., Lijtens, M., Weelij, D. R., Garcia-Vallejo, J. J., van Vliet, S. J., Saeland, E., van Kooyk, Y. (2009) Characterization of murine MGL1 and MGL2 C-type lectins: distinct glycan specificities and tumor binding properties. *Mol. Immunol.* **46**, 1240–1249.
104. Van Vliet, S. J., Saeland, E., van Kooyk, Y. (2008) Sweet preferences of MGL: carbohydrate specificity and function. *Trends Immunol.* **29**, 83–90.
105. Van Vliet, S. J., Gringhuis, S. I., Geijtenbeek, T. B., van Kooyk, Y. (2006) Regulation of effector T cells by antigen-presenting cells via interaction of the C-type lectin MGL with CD45. *Nat. Immunol.* **7**, 1200–1208.
106. Van Vliet, S. J., van Liempt, E., Geijtenbeek, T. B., van Kooyk, Y. (2006) Differential regulation of C-type lectin expression on tolerogenic dendritic cell subsets. *Immunobiology* **211**, 577–585.
107. Van Liempt, E., van Vliet, S. J., Engering, A., Garcia Vallejo, J. J., Bank, C. M., Sanchez-Hernandez, M., van Kooyk, Y., van Die, I. (2007) *Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol. Immunol.* **44**, 2605–2615.
108. Van Sorge, N. M., Bleumink, N. M., van Vliet, S. J., Saeland, E., van der Pol, W. L., van Kooyk, Y., van Putten, J. P. (2009) N-glycosylated proteins and distinct lipooligosaccharide glycoforms of *Campylobacter jejuni* target the human C-type lectin receptor MGL. *Cell. Microbiol.* **11**, 1768–1781.
109. Takada, A., Fujioka, K., Tsuiji, M., Morikawa, A., Higashi, N., Ebihara, H., Kobasa, D., Feldmann, H., Irimura, T., Kawaoka, Y. (2004) Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J. Virol.* **78**, 2943–2947.
110. Matsuno, K., Kishida, N., Usami, K., Igarashi, M., Yoshida, R., Nakayama, E., Shimojima, M., Feldmann, H., Irimura, T., Kawaoka, Y., Takada, A. (2010) Different potential of C-type lectin-mediated entry between Marburg virus strains. *J. Virol.* **84**, 5140–5147.
111. Usami, K., Matsuno, K., Igarashi, M., Denda-Nagai, K., Takada, A., Irimura, T. (2011) Involvement of viral envelope GP2 in Ebola virus entry into cells expressing the macrophage galactose-type C-type lectin. *Biochem. Biophys. Res. Commun.* **407**, 74–78.
112. Feinberg, H., Mitchell, D. A., Drickamer, K., Weis, W. I. (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* **294**, 2163–2166.
113. Mitchell, D. A., Fadden, A. J., Drickamer, K. (2001) A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. *J. Biol. Chem.* **276**, 28939–28945.
114. Guo, Y., Feinberg, H., Conroy, E., Mitchell, D. A., Alvarez, R., Blixt, O., Taylor, M. E., Weis, W. I., Drickamer, K. (2004) Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat. Struct. Mol. Biol.* **11**, 591–598.
115. Engering, A., Geijtenbeek, T. B., van Vliet, S. J., Wijers, M., van Liempt, E., Demareux, N., Lanzavecchia, A., Franssen, J., Figdor, C. G., Piguet, V., van Kooyk, Y. (2002) The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J. Immunol.* **168**, 2118–2126.
116. Geijtenbeek, T. B., Kwon, D. S., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Middel, J., Cornelissen, I. L., Nottet, H. S., KewalRamani, V. N., Littman, D. R., Figdor, C. G., van Kooyk, Y. (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100**, 587–597.
117. Geijtenbeek, T. B., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Adema, G. J., van Kooyk, Y., Figdor, C. G. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **100**, 575–585.
118. Soilleux, E. J., Morris, L. S., Leslie, G., Chehimi, J., Luo, Q., Levroney, E., Trowsdale, J., Montaner, L. J., Doms, R. W., Weissman, D., Coleman, N., Lee, B. (2002) Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. *J. Leukoc. Biol.* **71**, 445–457.
119. Khoo, U. S., Chan, K. Y., Chan, V. S., Lin, C. L. (2008) DC-SIGN and L-SIGN: the SIGNs for infection. *J. Mol. Med.* **86**, 861–874.
120. Bergman, M. P., Engering, A., Smits, H. H., van Vliet, S. J., van Bodegraven, A. A., Wirth, H. P., Kapsenberg, M. L., Vandenbroucke-Grauls, C. M., van Kooyk, Y., Appelmek, B. J. (2004) *Helicobacter pylori* modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. *J. Exp. Med.* **200**, 979–990.
121. Smits, H. H., Engering, A., van der Kleij, D., de Jong, E. C., Schipper, K., van Capel, T. M., Zaat, B. A., Yazdanbakhsh, M., Wierenga, E. A., van Kooyk, Y., Kapsenberg, M. L. (2005) Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J. Allergy Clin. Immunol.* **115**, 1260–1267.
122. Gringhuis, S. I., den Dunnen, J., Lijtens, M., van der Vlist, M., Geijtenbeek, T. B. (2009) Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat. Immunol.* **10**, 1081–1088.
123. Tassaneerithee, B., Burgess, T. H., Granelli-Piperino, A., Trumppfeller, C., Finke, J., Sun, W., Eller, M. A., Pattanapanyasat, K., Sarasombath, S., Birx, D. L., Steinman, R. M., Schlesinger, S., Marovich, M. A. (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J. Exp. Med.* **197**, 823–829.
124. Davis, C. W., Nguyen, H. Y., Hanna, S. L., Sanchez, M. D., Doms, R. W., Pierson, T. C. (2006) West Nile virus discriminates between DC-SIGN and DC-SIGNR for cellular attachment and infection. *J. Virol.* **80**, 1290–1301.
125. Simmons, G., Reeves, J. D., Grogan, C. C., Vandenberghe, L. H., Baribaud, F., Whitbeck, J. C., Burke, E., Buchmeier, M. J., Soilleux, E. J., Riley, J. L., Doms, R. W., Bates, P., Pohlmann, S. (2003) DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* **305**, 115–123.
126. Marzi, A., Moller, P., Hanna, S. L., Harrer, T., Eisemann, J., Steinkasserer, A., Becker, S., Baribaud, F., Pohlmann, S. (2007) Analysis of the interaction of Ebola virus glycoprotein with DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin) and its homologue DC-SIGNR. *J. Infect. Dis.* **196** (Suppl. 2), S237–S246.
127. Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L., Delgado, R. (2002) C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J. Virol.* **76**, 6841–6844.
128. Marzi, A., Gramberg, T., Simmons, G., Moller, P., Rennekamp, A. J., Krumbiegel, M., Geier, M., Eisemann, J., Turza, N., Saunier, B., Steinkasserer, A., Becker, S., Bates, P., Hofmann, H., Pohlmann, S. (2004) DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. *J. Virol.* **78**, 12090–12095.
129. North, S. J., Huang, H. H., Sundaram, S., Jang-Lee, J., Etienne, A. T., Trollope, A., Chalabi, S., Dell, A., Stanley, P., Haslam, S. M. (2010) Glycomics profiling of Chinese hamster ovary cell glycosylation mutants reveals N-glycans of a novel size and complexity. *J. Biol. Chem.* **285**, 5759–5775.
130. Stanley, P., Sudo, T., Carver, J. P. (1980) Differential involvement of cell surface sialic acid residues in wheat germ agglutinin binding to parental and wheat germ agglutinin-resistant Chinese hamster ovary cells. *J. Cell Biol.* **85**, 60–69.
131. Sauter, N. K., Bednarski, M. D., Wurzburg, B. A., Hanson, J. E., Whitesides, G. M., Skehel, J. J., Wiley, D. C. (1989) Hemagglutinins from two influenza virus variants bind to sialic acid derivatives with millimolar dissociation constants: a 500-MHz proton nuclear magnetic resonance study. *Biochemistry* **28**, 8388–8396.
132. Lees, W. J., Spaltenstein, A., Kingery-Wood, J. E., Whitesides, G. M. (1994) Polyacrylamides bearing pendant  $\alpha$ -sialoside groups strongly inhibit agglutination of erythrocytes by influenza A virus: multivalency and steric stabilization of particulate biological systems. *J. Med. Chem.* **37**, 3419–3433.
133. Blum, J. S., Stahl, P. D., Diaz, R., Fiani, M. L. (1991) Purification and characterization of the D-mannose receptor from J774 mouse macrophage cells. *Carbohydr. Res.* **213**, 145–153.
134. Diment, S., Leech, M. S., Stahl, P. D. (1987) Generation of macrophage variants with 5-azacytidine: selection for mannose receptor expression. *J. Leukoc. Biol.* **42**, 485–490.
135. Wileman, T. E., Lennartz, M. R., Stahl, P. D. (1986) Identification of the macrophage mannose receptor as a 175-kDa membrane protein. *Proc. Natl. Acad. Sci. USA* **83**, 2501–2505.
136. Kawasaki, T., Ii, M., Kozutsumi, Y., Yamashina, I. (1986) Isolation and characterization of a receptor lectin specific for galactose/N-acetylgalactosamine from macrophages. *Carbohydr. Res.* **151**, 197–206.
137. Snyder, G. A., Ford, J., Torabi-Parizi, P., Arthos, J. A., Schuck, P., Colonna, M., Sun, P. D. (2005) Characterization of DC-SIGN/R interaction with human immunodeficiency virus type 1 gp120 and ICAM molecules

- favors the receptor's role as an antigen-capturing rather than an adhesion receptor. *J. Virol.* **79**, 4589–4598.
138. Nguyen, D. G., Hildreth, J. E. (2003) Involvement of macrophage mannose receptor in the binding and transmission of HIV by macrophages. *Eur. J. Immunol.* **33**, 483–493.
  139. Pokidysheva, E., Zhang, Y., Battisti, A. J., Bator-Kelly, C. M., Chipman, P. R., Xiao, C., Gregorio, G. G., Hendrickson, W. A., Kuhn, R. J., Rossman, M. G. (2006) Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell* **124**, 485–493.
  140. Lozach, P. Y., Burleigh, L., Staropoli, I., Navarro-Sanchez, E., Harriague, J., Virelizier, J. L., Rey, F. A., Despres, P., Arenzana-Seisdedos, F., Amara, A. (2005) Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J. Biol. Chem.* **280**, 23698–23708.
  141. Navarro-Sanchez, E., Altmeyer, R., Amara, A., Schwartz, O., Fieschi, F., Virelizier, J. L., Arenzana-Seisdedos, F., Despres, P. (2003) Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep.* **4**, 723–728.
  142. Baribaud, F., Doms, R. W., Pohlmann, S. (2002) The role of DC-SIGN and DC-SIGNR in HIV and Ebola virus infection: can potential therapeutics block virus transmission and dissemination? *Expert Opin. Ther. Targets* **6**, 423–431.
  143. Pohlmann, S., Baribaud, F., Doms, R. W. (2001) DC-SIGN and DC-SIGNR: helping hands for HIV. *Trends Immunol.* **22**, 643–646.
  144. Pohlmann, S., Baribaud, F., Lee, B., Leslie, G. J., Sanchez, M. D., Hiebenthal-Millow, K., Munch, J., Kirchhoff, F., Doms, R. W. (2001) DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J. Virol.* **75**, 4664–4672.
  145. Geijtenbeek, T. B., Koopman, G., van Duijnhoven, G. C., van Vliet, S. J., van Schijndel, A. C., Engering, A., Heeney, J. L., van Kooyk, Y. (2001) Rhesus macaque and chimpanzee DC-SIGN act as HIV/SIV gp120 trans-receptors, similar to human DC-SIGN. *Immunol. Lett.* **79**, 101–107.
  146. Cormier, E. G., Durso, R. J., Tsamis, F., Boussemart, L., Manix, C., Olson, W. C., Gardner, J. P., Dragic, T. (2004) L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **101**, 14067–14072.
  147. Lai, W. K., Sun, P. J., Zhang, J., Jennings, A., Lalor, P. F., Hubscher, S., McKeating, J. A., Adams, D. H. (2006) Expression of DC-SIGN and DC-SIGNR on human sinusoidal endothelium: a role for capturing hepatitis C virus particles. *Am. J. Pathol.* **169**, 200–208.
  148. Lozach, P.Y., Lortat-Jacob, H., de Lacroix de Lavalette, A., Staropoli, I., Foug, S., Amara, A., Houles, C., Fieschi, F., Schwartz, O., Virelizier, J.L., Arenzana-Seisdedos, F., Altmeyer, R. (2003) DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J. Biol. Chem.* **278**, 20358–20366.
  149. Halary, F., Amara, A., Lortat-Jacob, H., Messerle, M., Delaunay, T., Houles, C., Fieschi, F., Arenzana-Seisdedos, F., Moreau, J. F., Dechanet-Merville, J. (2002) Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity* **17**, 653–664.
  150. Martina, B. E., Koraka, P., van den Doel, P., Rimmelzwaan, G. F., Haagmans, B. L., Osterhaus, A. D. (2008) DC-SIGN enhances infection of cells with glycosylated West Nile virus in vitro and virus replication in human dendritic cells induces production of IFN- $\alpha$  and TNF- $\alpha$ . *Virus Res.* **135**, 64–71.
  151. Han, D. P., Lohani, M., Cho, M. W. (2007) Specific asparagine-linked glycosylation sites are critical for DC-SIGN- and L-SIGN-mediated severe acute respiratory syndrome coronavirus entry. *J. Virol.* **81**, 12029–12039.
  152. Burnet, F. (1960) *Principles of Animal Virology*, Academic, New York, NY, USA.
  153. Reading, P. C., Whitney, P. G., Pickett, D. L., Tate, M. D., Brooks, A. G. (2010) Influenza viruses differ in ability to infect macrophages and to induce a local inflammatory response following intraperitoneal injection of mice. *Immunol. Cell Biol.* **88**, 641–650.
  154. Tate, M. D., Pickett, D. L., van Rooijen, N., Brooks, A. G., Reading, P. C. (2010) Critical role of airway macrophages in modulating disease severity during influenza virus infection of mice. *J. Virol.* **84**, 7569–7580.
  155. Glaser, L., Conenello, G., Paulson, J., Palese, P. (2007) Effective replication of human influenza viruses in mice lacking a major  $\alpha$ 2,6 sialyltransferase. *Virus Res.* **126**, 9–18.
  156. Ning, Z. Y., Luo, M. Y., Qi, W. B., Yu, B., Jiao, P. R., Liao, M. (2009) Detection of expression of influenza virus receptors in tissues of BALB/c mice by histochemistry. *Vet. Res. Commun.* **33**, 895–903.

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**KEY WORDS:**

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