Chapter 3
The DAP12-Associated Myeloid C-Type Lectin 5A (CLEC5A)

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Abstract Innate immunity is the first line of host defense mechanism against pathogen invasion. In order to recognize various pathogen-associated molecular patterns, myeloid cells express abundant innate immunity receptors on cell surface to recognize diverse pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs) are the most well-characterized innate immunity receptors for pattern recognition, and activation of TLRs triggers the MyD88- and TRIF-dependent pathways to induce the secretion of pro-inflammatory cytokines and interferons (Akira and Takeda 2004; Athman and Philpott 2004). In addition to TLRs, natural killer cells and myeloid cells, which are the key players in innate immunity, recognize glycan and non-glycan structures on pathogen surface via the C-type lectin receptors (denoted as CLRs), which are the most abundant lectins in human genome. Among the myeloid CLRs, the spleen tyrosine kinase (Syk)-coupled C-type lectin receptors (Syk-CLRs) have been shown to play critical roles in host defense against pathogen invasion (Osorio et al. 2011; Sancho et al. 2012). Here, we discuss the potential roles of CLEC5A, also known as "myeloid DAP12-associating lectin-1 (MDL-1)," in host defense and autoimmunity. We would also discuss the impact of dual recognition by CLEC5A and TLRs in future study of host-pathogen interaction.

Keywords TLR • Syk • CLR • Lectins • Innate immunity • PAMPs • ITAM

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S. Yamasaki (ed.), *C-Type Lectin Receptors in Immunity*,
DOI 10.1007/978-4-431-56015-9_3

3.1 The Syk-Coupled C-Type Lectins (Syk-CLRs) in Human Genome

There are approximately 150 proteins with "C-type lectin-like domain" (denoted as CTLD family) classified into 17 groups in human genome (Varki et al. 2009). All the Syk-CLRs are type II transmembrane proteins belonging to either group II or group V of CTLD family. The type II C-type lectins contain four Syk-CLRs [CLEC4B (DCAR2), CLEC4C (BDCA-2), CLEC4E (Mincle), and CLEC6A (Dectin-2)]. In addition, the type V C-type lectins (also denoted as NK receptor-like lectin) contains four Syk-CLRs [CLEC2, CLEC5A (MDL-1), CLEC7A (Dectin-1), CLEC9A (CD370)] and 11 NK receptors [CLEC5B (NKR-P1A, CD161), CLEC5C (KLRF1), CLEC15B (KLRG2), MAFA (KLRG1), NKG2A (KLRC1, CD159a), NKG2B (KLRC1), NKG2C (KLRC2, CD159c), NKG2D (CD314), NKG2E (KLRC3), NKG2F (KLRC4), NKG2I (KLRE1)].

3.1.1 Syk-CLRs with ITAM Motif

CLEC7A (Dectin-1) and CLEC9A (CD370) possess a cytoplasmic immunoreceptor tyrosine-based activation-like YxxL motif (ITAM, YxxL-(X)10-12-YxxL) that can recruit Syk kinase. CLEC2 has a YxxL motif in its cytoplasmic tail, which is crucial for CLEC2-mediated signal transduction. This sequence is called hemi-ITAM, since its cytoplasmic domain only has one YxxL motif. CLEC1 contains a tyrosine residue in the sequence YSST, which may represent a novel signaling motif.

3.1.2 Syk-CLR Associated with Adaptor Fc Receptor Gamma-Chain (FcRγ)

All group II Syk-CLRs transduce signal via association with the ITAM-containing adaptor protein FcRγ: CLEC4B (DCAR2), CLEC4C (BDCA-2), CLEC4E (Mincle), and CLEC6A (Dectin-2).

3.1.3 Syk-CLR Associated with Adaptor DNAX-Associated Protein 12 (DAP12)

There are 12 DAP12-associated proteins in the group V CTLD family. While 11 members belong to NK receptors, the myeloid C-type lectin CLEC5A (MDL-1) is abundantly expressed in monocytes, macrophages, and

polymorphonuclear cells (PMNs), but not in NK cells, dendritic cells, lymphocytes, or platelets. While human CLEC5A is only reported to associate with DAP12 (Chen et al. 2008), mouse CLEC5A is able to associate with DAP12 (Bakker et al. 1999) (in monocytes, macrophages, PMNs) and DAP10 (Inui et al. 2009) (in osteoclast).

3.2 CLEC5A (MDL-1) and Its Known Ligands

There are four myeloid Syk-CLRs [CLEC7A (Dectin-1), CLEC6A (Dectin-2), CLEC4E (Mincle), and CLEC5A (MDL-1)] involved in recognition of fungi (Taylor et al. 2007; Saijo et al. 2007; Wells et al. 2008), mycobacterial glycolipid (Ishikawa et al. 2009), virus (Chen et al. 2008, 2012), and bacteria (Rabes et al. 2015). Compared to the other three myeloid Syk-CLRs, the functions of CLEC5A are relatively un-explored yet. In this chapter, we will discuss the potential functions of CLEC5A in host-pathogen interaction, autoimmunity, and septic shock.

3.2.1 Discovery of CLEC5A

CLEC5A, initially named as "myeloid DAP12-associating lectin-1 (MDL-1)," was cloned in Dr. Lanier's group by indirect expression cloning in 1999 (Bakker et al. 1999). DAP12 is located in the cytosol without the presence of DAP12-associating receptor, while it was translocated to cell surface when cells where co-transfected with DAP12 and DAP12-associating proteins. By transfecting mouse cDNA library with flag-tagged DAP12 cDNA, they identified CLEC5A, a novel cDNA clone, associated with DAP12 (Bakker et al. 1999). Cross-linking of CLEC5A is able to induce calcium mobilization, suggesting CLEC5A is able to activate cells via DAP12-mediated signaling. Furthermore, CLEC5A is expressed in the myeloid lineage, including monocytes, macrophages, neutrophils, microglia, and osteoclasts, but not in monocyte-derived dendritic cells. The specific expression of CLEC5A in myeloid lineage is further confirmed by the observation that CLEC5A is a novel PU.1 transcriptional target during myeloid differentiation (Batliner et al. 2011). Furthermore, crystallization and X-ray diffraction analysis of human CLEC5A revealed that CLEC5A forms a homodimer on cell surface (Watson and O'Callaghan 2010). However, the ligand and function of CLEC5A were unknown until the discovery of CLEC5A as the dengue recognition receptor (Chen et al. 2008).

NCBI (AAI13100) 188 aa(CLEC5A):

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  1 MNWHMIISGL IVVVLKVVGM TLFLLYFPQI FNKSNDGFTT TRSYGTVSQI FGSSSPSPNG
 61 FITTRSYGTV CPKDWEFYQA RCFFLSTSES SWNESRDFCK GKGSTLAIVN TPEKLKFLQD
121 ITDAEKYFIG LIYHREEKRW RWINNSVFNG NVTNQNQNFN CATIGLTKTF DAASCDISYR
181 RICEKNAK
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NCBI (NP_037384) 165 aa(CLEC5A_S)

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  1 MNWHMIISGL IVVVLKVVGM TLFLLYFPQI FNKSNDGFTT TRSYGTVCPK DWEFYQARCF
 61 FLSTSESSWN ESRDFCKGKG STLAIVNTPE KLKFLQDITD AEKYFIGLIY HREEKRWRWI
121 NNSVFNGNVT NQNQNFNCAT IGLTKTFDAA SCDISYRRIC EKNAK
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Fig. 3.1 Alignment of human CLEC5A and its variant (CLEC5A_S). Human CLEC5A is a 188 a.a. polypeptide belonging to type II transmembrane protein. The transmembrane domain is located in a.a. 3–25, the neck region is located in a.a. 26–70, and the CTLD is located in a.a. 71–188. The "K_{16}" is critical for association with DAP12. The CLEC5A_S is short of 23 a.a. ($_{48}$SQIFGSSSPSPNGFITTRSYGTV$_{70}$) in the neck region

3.2.2 Length Polymorphism of CLEC5A

The full-length CLEC5A cDNA encodes a 188 a.a. peptide belonging to type II transmembrane protein with a putative N-linked glycosylation site in the extracellular domain. In addition, the alternative splicing variant CLEC5A_S1 (165 a.a.) was found in human peripheral blood cells. The CLEC5A_S1 lacks 23 a.a. ($_{48}$SQIFGSSSPSPNGFITTRSYGTV$_{70}$) in the neck region (Fig. 3.1).

It is interesting to note that length polymorphism in the neck region was also found in other CLRs. The 23 a.a. imperfect repeat segment (QNLTQLKAAVGEL-SEKSKLQEIY) is responsible for the length polymorphism of CLEC4L (DC-SIGN/CD209) and CLEC4M (DC-SIGNR/CD299). The predominant form of CLEC4L (DC-SIGN/CD209) and CLEC4M (DC-SIGNR/CD299) contains 7 repeats of largely conserved 23 amino acid segment. It has been shown that the neck region of CLR is responsible for homo-oligomerization and thus allows the CLR to bind multivalent ligands with high avidity, thus the length polymorphism in neck region would affect its binding affinity and specificity to their ligands. This speculation is supported by the observation that variable repeats in the neck region of CLEC4M (DC-SIGNR/CD299) affect its interaction with HIV-1 (Gramberg et al. 2006) and associates with susceptibility to HCV infection (Nattermann et al. 2006). The crystallography shows that CLEC5A forms homodimer, and twisting of neck region can elicit conformation change to activate CLEC5A (Watson et al. 2011). Whether the length polymorphism in CLEC5A neck region has significant impact on ligand binding needs to be further characterized.

3.2.3 *CLEC5A Is the Pattern Recognition Receptor to Flaviviruses*

Even though the other three myeloid lectins (CLEC6A, CLEC7A, and CLEC4E) have been shown to interact with various microbes, the ligand of CLEC5A was unknown until Chen et al. showed that CLEC5A interacts with dengue virus directly and plays a critical role in the pathogenesis of dengue hemorrhagic fever and dengue shock syndrome (Chen et al. 2008; Hsu et al. 2009).

Previous studies on host-virus interaction focused on the identification of "viral entry receptor." In contrast, whether virus can bind and activate innate immunity receptor via the "pathogen-associated molecular patterns" (PAMPs) has never been tested until we showed that CLEC5A can interact with members of flaviviruses. Similar to the outer most structures of microorganisms, most of the viral envelope proteins are also glycosylated. Thus, the distinct distribution of glycans on either the helical or icosahedral structures of each virus would be ideal PAMPs to bind and activate pattern recognition receptors. To address this question, we cloned more than 30 pattern recognition receptors, including C-type lectins, Siglecs, TLRs, TREMs (triggering receptor expressed on myeloid cells), and TREM-like receptors (TLTs) (Chen et al. 2008). The extracellular receptors of these receptors were fused with the Fc portion of human IgG1 and the recombinant receptor. Fc fusion proteins were expressed on mammalian 293 T cells. The conformation of these recombinant proteins was examined by sugar completion assay to confirm their glycan-binding specificity (Hsu et al. 2009).

By the ELISA-based binding assay, we found that the intact dengue virions bind to CLEC4L (DC-SIGN/CD209), CLEC4M (DC-SIGNR/CD299), and CLEC5A, specifically. Moreover, both live DV and UV-inactivated DV can induce DAP12 phosphorylation via CLEC5A in human macrophages, suggesting DV can trigger DAP12 phosphorylation via CLEC5A without replication. These results suggest that virions can act as ligands to bind and activate CLEC5A directly. We further clone CLEC5A_S (the short form of CLEC5A) and compare the binding specificity of CLEC5A.Fc and CLEC5A_S_Fc binding to DV, JEV, and WNV. Similar binding results were found between CLEC5A.Fc and CLEC5A_S_Fc, suggesting the neck domain length polymorphism did not have significant influence of binding specificity to members of flaviviruses. In addition, we also find that CLEC5A binds specifically to influenza virus H5N1, but not H1N1, specifically.

To further understand how CLEC5A interacts with dengue virus, we developed a novel detection method based on nanostructured hemisphere-based biosensor for lectin-virion interaction (Fig. 3.2) (Tung et al. 2014). Since glycans on icosahedral virion is not on a flat plan, the interaction between viral glycans and immobilized lectin.Fc is limited. The weak interaction between lectins and viral glycans makes it extremely difficult in determining the **stringency** of **washing** to discriminate specific vs. nonspecific interaction by conventional ELISA. To overcome this problem, we immobilized the lectin.Fc fusion proteins on the nanostructured hemisphere surface to form multiple-valence binding between lectins and virions.

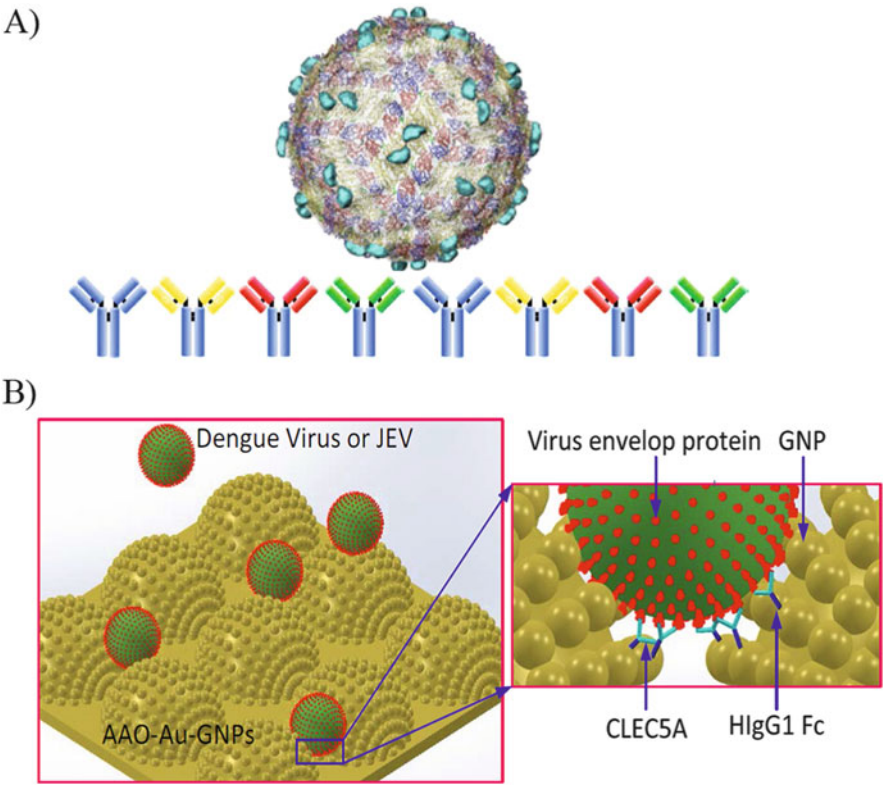

Fig. 3.2 Interaction of virions with immobilized lectin.Fc fusion protein. (**a**) The interaction between immobilized recombinant lectin.Fc on flat surface with glycans on virus surface is limited. (**b**) The nanostructured hemisphere creates a three-dimensional space to all the multiple-valence binding between immobilized lectin.Fc and glycans on viral surfaces (Figure b is provided by Dr. Gou-Jen Wang and Yen-Ting Wang (National Chung-Hsing University))

By comparing the binding affinity between the wild-type (WT) and mutant deficient of glycosylation site (Asn67 mutant and Asn153 mutant), CLEC5A was found to interact with Asn153, while CLEC4L (DC-SIGN/CD209) interacts with Asn153 of dengue virus (DV) envelope protein (un-published data). Since crystallography has shown that DC-SIGN interacts with the glycans on Asn153 (Pokidysheva et al. 2006), this observation suggests that DV can bind to both CLEC5A and DC-SIGN via glycans on Asn67 and Asn153, respectively, to trigger downstream signaling pathways.

3.3 CLEC5A in Flaviviral Infections

The *Flavivirus* genus includes the mosquito-borne dengue, Japanese encephalitis, and yellow fever viruses (Mukhopadhyay et al. 2005), and infections of flaviviruses can result in diverse clinical syndromes such as encephalitis, hemorrhagic fever, and shock syndrome (Mackenzie et al. 2004). There are four serotypes of DV, which can give rise to severe hemorrhagic syndrome (dengue hemorrhagic fever/DHF) and capillary leakage induced-hypovolemic shock (dengue shock syndrome/DSS) (Wilder-Smith and Schwartz 2005). Dengue is a major public health problem, with ~50 million people infected each year (of whom ~20,000 die) and ~2.5 billion people worldwide being at risk of infection. On the other hand, the Japanese encephalitis virus (JEV) serological group, which includes West Nile virus (WNV) and St. Louis encephalitis virus, is a major contributor to the occurrence of viral encephalitis worldwide (Weaver and Barrett 2004), with 50,000 new cases and 15,000 deaths per annum (Hollidge et al. 2010). Myeloid cells, including monocytes, macrophages, and dendritic cells, are the major targets of flaviviruses, and overactivation of macrophages by DV causes massive release for cytokines known as "cytokine storm," which is believed to be responsible for DHF and DSS. However, how DV activates macrophages to trigger release of inflammatory cytokines is unknown till we showed that CLEC5A is critical for DV-induced lethality in mouse model (Chen et al. 2008).

3.3.1 CLEC5A in the Pathogenesis of DV Infection

Dengue is a mosquito-borne infection caused by four serotypes of *dengue virus* (DV1-4) and is currently the most common arboviral disease worldwide (Wilder-Smith and Schwartz 2005). Primary infection with any of the four DV serotypes typically results in mild dengue fever (DF) and provides lifelong immunity to the infecting strain. However, secondary infection with different DV serotypes is associated with increased risk of developing DHF (characterized by thrombocytopenia and capillary leakage) and can progress to life-threatening hypovolemic DSS (Wilder-Smith and Schwartz 2005). The pathogenesis of DHF/DSS remains unclear, but massive cytokine secretion (cytokine storm) is believed to be one of the major contributory factors (Varki et al. 2009). Unfortunately, no DV-specific therapies or vaccines are available (Wilder-Smith and Schwartz 2005).

Since wild-type mice are resistant to DV infection, we utilized the STAT1-deficient mice as a model system to demonstrate the role of CLEC5A in flaviviral infection. We found that DV induces cytokine storms and causes systemic permeability leakage, subcutaneous hemorrhage, and systemic shock syndrome (Chen et al. 2008). Blockade of CLEC5A by antagonistic monoclonal antibodies (mAbs) suppressed DV-induced cytokine storm and rescued mice from DV-induced lethality (Chen et al. 2008). To further evaluate the critical genes involved in DHF,

Gomez et al. analyzed the gene expression in peripheral blood mononuclear cells of dengue patients using the "support vector machines" (SM) algorithm. They found that CLEC5A was associated with disease severity among the 28 dengue patients they analyzed (Gomes et al. 2010). Furthermore, genetic variations at CLEC5A were shown to increase the risk and regulate TNF secretion in dengue severity among Brazilians (Xavier-Carvalho et al. 2013). These observations demonstrated the critical role of CLEC5A in the pathogenesis of DHF and DSS.

3.3.2 CLEC5A in the Pathogenesis of JEV Infection

JEV is the most prevalent cause of encephalitis worldwide, even though both inactivated (Hoke et al. 1988) and live-attenuated JEV vaccines (Xin et al. 1988) have been used in Asia for decades. In fact, these vaccines are not completely effective against all the clinical isolates (Ku et al. 1994), and there are still ~35,000 reported cases of Japanese encephalitis (JE) and 10,000 deaths each year (Olsen et al. 2010). Unlike DHF and DSS, JE victims experience permanent neuropsychiatric sequelae, including persistent motor defects and severe cognitive and language impairments (Mackenzie et al. 2004). However, the molecular mechanism for the pathogenesis of JEV infection is still unclear.

We found that JEV also interacted with the CLEC5A and CLEC5A_S directly. Moreover, JEV can infect and activate microglia to induce the secretion of pro-inflammatory cytokines and neurotoxic substances. In contrast, blockade of CLEC5A by antagonistic mAb can suppress the release of pro-inflammatory cytokines and neurotoxic substances (Chen et al. 2012). Injection of antagonistic anti-CLEC5A mAbs maintains the intact of blood brain barrier and prevents JEV-induced permeability change and neurotoxicity. The protective effect is not from inhibiting JEV infection to neuronal cells, but via the inhibition of neuronal inflammation and cell infiltration from peripheral blood. Notably, mice receiving anti-CLEC5A mAbs are still able to develop protective cellular and humoral immunity against JEV infection. This observation demonstrated that blockade of CLEC5A during acute infection does not interfere the development of host immune response against JEV (Chen et al. 2012).

3.4 CLEC5A in Shock Syndrome

It is interesting to find that CLEC5A-positive cells play critical role in concanavalin A (ConA)-induced liver injury and lethal shock in mouse model system (Cheung et al. 2011). ConA-induced hepatic injury closely resembles the pathophysiology of T cell-mediated liver diseases; it therefore has been used extensively as an animal model for autoimmune and viral hepatitis (Tiegs et al. 1992). After ConA injection, massive CLEC5A-positive cells were found to infiltrate into mouse liver. The

expression of CLEC5A from high to low was found in $CD11b^{+}Ly6G^{+}Ly6C^{lo}$ cells, followed by the $CD11b^{+}Ly6G^{-}Ly6C^{hi}$ and $CD11b^{+}Ly6G^{-}Ly6C^{lo}$ cells. Flow cytometry analysis further demonstrated that the CLEC5A-positive cells express CD33 and MHC class I, but not CD34 or MHC class II, suggesting the ConA-induced CLEC5A-positive cells in liver are the immature myeloid cells. Interestingly, activation of CLEC5A-positive cells by agonistic anti-CLEC5A mAb induced NO and TNF-α release in vitro, while injection of agonistic anti-CLEC5A mAb or DV into ConA-treated mice caused lethal shock in wild-type mice within 60 min and 24 h, respectively. This observation suggests that CLEC5A-mediated signaling may contribute to lethal shock syndrome during systemic inflammatory response syndrome (SIRS).

3.5 CLEC5A in Autoimmunity

It is interesting to find that CLEC5A is not only limited to host defense against flaviviral infection but also involved in the development of autoimmune diseases (Joyce-Shaikh et al. 2010; Chen et al. 2014). CLEC5A is highly expressed in mouse bone marrow and bone tissues, and highest levels of CLEC5A were found in the $CD11b^{+}Ly6G^{+}$ cells. In the collagen antibody-induced arthritis (CAIA) model, the $CD11b^{+}Ly6G^{+}$ cells increased up to 50 % and 40 % in bone marrow and peripheral blood after injection of anti-collagen mAb, and co-injection of arthrogenic and anti-CLEC5A mAb exacerbated disease severity. In addition, CLEC5A-deficient mice are resistant to collagen-induced arthritis (CIA), and this observation provides direct evidence that CLEC5A is critical for the pathogenesis of autoimmune arthritis. Since injection of recombinant CLEC5A.Fc is able to suppress bone erosion in CIA mice model, the CLEC5A-positive cells may recognize yet-identified endogenous ligands expressed in synovia to cause tissue damage.

To further understand the role of CLEC5A in autoimmune arthritis, we examine the expression of CLEC5A in peripheral blood isolated from arthritis patients (Chen et al. 2014). We found that the number of CLEC5A-positive monocyte is much higher in patients with active (53.6 %) and inactive (31 %) rheumatoid arthritis than osteoarthritis (27.9 %) and healthy control (21.2 %). Moreover, CLEC5A expression level in synovia was positively correlated with parameters of disease activity, articular damage, and levels of pro-inflammatory cytokines (Chen et al. 2014).

3.6 Cross Talk Between CLEC5A and TLRs in Host Defense Mechanism

Several intracellular receptors (TLR 3, 7, 8, 9) (Mikula et al. 2010) and sensors (MDA5, RIG-I, and AIM) (Kanneganti 2010) have been shown to be responsible for virus-induced inflammatory reactions via recognition of viral nucleic acids. In addition, surface receptors TLR2 (Kim et al. 2012) and TLR4 (Kurt-Jones et al. 2000) are also involved in host recognition to herpes simplex virus (HSV) and respiratory syncytial virus, respectively. However, whether intact virions physically interact with TLR2 and TLR4 is still unknown. It is interestingly observed that even HSV-1 can signal via the TLR2, this receptor does not mediate recognition of HSV glycoproteins (Reske et al. 2008). This observation suggests that TLR2 may associate with other receptor on cell surface to recognize the glycoproteins on HSV. We have shown that CLEC5A physically interacts with flaviviruses, and flaviviruses can activate CLEC5A directly to trigger downstream signaling. This observation suggests the possibility that virus may co-activate CLEC5A with members of TLRs to trigger downstream signaling.

3.6.1 Cross Talk Between CLEC5A and TLR4 in NALP3 Inflammasome Activation

Fever is one of the most typical symptoms for all DV patients, but how DV causes the severe high fever in patients is not clear, even though human macrophages have been regarded as the major source of inflammatory cytokines (Chen and Wang 2002) and the major target cells for DV replication (Jessie et al. 2004). Macrophages are heterogeneous, and their phenotypes depend on the presence of cytokines and other factors during its differentiation process. While M-CSF is essential for the development of "resting macrophages" (denoted as M-Mϕ), high level of GM-CSF is critical for monocyte differentiation into "inflammatory macrophages" (also denoted as GM-Mϕ) during inflammatory reaction (Wu et al. 2013a). Since IL-1β and TNF-α are the most potent endogenous pyrogens (Netea et al. 2000), we are interested to identify the source of these two cytokines from human macrophages. We found that DV can induce pro-inflammatory cytokines (TNF-α, IL-1β, and IL-18) from GM-Mϕ, but not M-Mϕ, even though the expression levels of CLEC5A and TLR4 are similar between these two populations (Wu et al. 2013b).

Unlike TNF-α, the production of IL-1α and IL-18 relies on the activation of inflammasome (Schroder and Tschopp 2010). Thus, we asked whether CLEC5A is also responsible for DV-induced inflammasome activation. While activation of CLEC5A induces low levels of IL-1β and IL-18, co-activation of CLEC5A and TLR4 induces high levels of IL-1β and IL-18 via NALP3 inflammasome. This observation implies that GM-Mϕ can produce high levels of endogenous pyrogens upon DV and gram-negative bacteria infection and can explain why high mortality

and morbidity were observed in dengue patients with concurrent bacterial infections (Lee et al. 2005).

3.6.2 Cross Talk Between CLEC5A and Other TLRs

It has been shown that DV can activate macrophages via CLEC5A and TLR7, and co-activation of CLEC5A and TLR enhances the secretion of pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, and IP-10, but not IFN-α (Chen et al. 2008). In contrast, knockdown of CLEC4L/DC-SIGN/CD209 did not affect DV-induced inflammatory cytokine release significantly. Interestingly, blockade of CLEC5A only suppressed the inflammatory cytokines, but not IFN-α. This observation suggests that co-activation of CLEC5A and TLR7 has synergistic effect on TNF-α production, and CLEC5A does not contribute to IFN-α secretion during DV infection. Thus, targeting CLEC5A is able to attenuate inflammatory reaction without attenuating antiviral immunity.

It has been shown that murine peritoneal neutrophils are the major source of IL-10 during sepsis by using the cecum ligation model (Kasten et al. 2010). Interestingly, co-activation of peritoneal neutrophils in Gr-1^{hi}/Ly6$G^{hi}$$CD^{155-/low}$ mice by immobilized anti-CLEC5A mAb, and TLR2 ligand Pam3 upregulated the secretion of IL-10 dramatically (Zhang et al. 2009). This observation demonstrates that the co-activation of CLEC5A and TLR2 is important for IL-10 secretion from murine peritoneal neutrophils. However, human neutrophils do not produce IL-10 (Davey et al. 2011); thus the effect of CLEC5A and TLR2 co-activation in human neutrophils needs to be further characterized in the future.

Above observations suggest that CLEC5A may form functional complex with specific TLRs in different cell lineages to generate combinatorial repertoires to recognize diverse PAMPs on pathogens. Genetic approach using CLEC5A and TLRs double knockout mice will be able to answer the significance of CLEC5A and TLRs in host defense mechanisms in the future.

3.7 Potential Functions of CLEC5A in Inflammatory Diseases

Previous studies have demonstrated that CLEC5A is involved in flaviviral infections, autoimmune arthritis, and aseptic shock syndrome. While flaviviruses have been shown to be CLEC5A exogenous ligands, the endogenous ligands of CLEC5A in autoimmunity are still unknown.

In addition to macrophages, CLEC5A is also upregulated in eosinophils and neutrophils. It has been shown that CLEC5A is upregulated in eosinophils isolated from asthma patients (Esnault et al. 2013), but whether CLEC5A is involved in

allergen recognition is still unknown. In contrast, the abundant expression of CLEC5A in neutrophils (Chen et al. 2008; Zhang et al. 2009) suggests that CLEC5A may play a critical role in neutrophil-mediated defense mechanisms, such as phagocytosis, cytotoxicity, and the formation of neutrophil extracellular traps (NETs). Since neutrophils are involved in host defense against bacteria, fungi, and other pathogens, CLEC5A may be also involved in host defense against nonviral pathogens. This argument is supported by the observation that CLEC5A binds preferentially to mannans and fucose. Since mannans are the essential components of fungal and bacterial cell walls, CLEC5A may also play important roles in host defense against bacterial and fungi. In contrast, the fucose-containing glycans play important role in blood transfusion reactions, leukocyte-endothelial adhesion, host-microbe interactions, and Notch receptor signaling. Moreover, alterations in the expression of fucosylated oligosaccharides were observed in cancer and atherosclerosis. Therefore, CLEC5A may be involved in the recognition of fucosylated glycans upregulated during microbe invasion, inflammation, cancer progression, and atherosclerosis. Thus, CLEC5A is not only critical for flaviviral infections and may be also involved in the pathogenesis of nonviral infections, transfusion, and autoimmunity.

Acknowledgements This work was supported by the National Science Council (NSC 103-2321-B-001-044 and NSC 103-2320-B-001-010-MY3) and Summit and Thematic Research Project (AS-101-TP-B06-2) of Academia Sinica.

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