Lectin Receptors Expressed on Myeloid Cells

GORDON D. BROWN¹ and PAUL R. CROCKER²
¹MRC Centre for Medical Mycology, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom; ²Division of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom

ABSTRACT Lectins recognize a diverse array of carbohydrate structures and perform numerous essential biological functions. Here we focus on only two families of lectins, the Siglecs and C-type lectins. Triggering of intracellular signaling cascades following ligand recognition by these receptors can have profound effects on the induction and modulation of immunity. In this chapter, we provide a brief overview of each family and then focus on selected examples that highlight how these lectins can influence myeloid cell functioning in health and disease.

INTRODUCTION Lectins, defined as proteins that recognize carbohydrates, perform numerous essential biological functions. Recognizing a diverse array of carbohydrate structures, vertebrate lectins have been subdivided into several structurally distinct families which can be located intracellularly (such as the intracellular M-type family of lectins, which function primarily in the glycoprotein secretory pathway), in the plasma membrane (such as some members of the C-type lectin and Siglec [sialic acid-binding immunoglobulin-type lectin] families, which are involved in pathogen recognition and immune regulation), or are secreted into the extracellular milieu (such as some members of the galectin family, which serve several homeostatic and immune functions) (Table 1). We will restrict our discussion here to selected myeloid- and plasma membrane-expressed members of only two families, the C-type lectins and Siglecs. We will provide a brief overview of each family and then focus on selected illustrative and detailed examples that highlight how these lectins influence myeloid cell functioning in health and disease. For an overview on the other lectin families, the reader is referred to an excellent website (http://www.imperial.ac.uk/research/animallectins/ctld/lectins.html).

SIGLECs Siglecs are a distinct subgroup of the immunoglobulin (Ig) superfamily that have evolved to use sialylated glycans as their predominant ligands (1). Siglecs have been mainly defined in mammalian species, but clear orthologs are also present in amphibia and fish (2). Siglec-like molecules have also been identified in streptococcal bacteria (3) and in an adenovirus capsid protein (4). In mammals, there are two subgroups of Siglecs: (i) sialoadhesin (Sn; Siglec-1), CD33 (Siglec-3), and Siglec-5, -7, -8, -9, -10, -11, -14, -15, -E, -F, and -G as well as Dectin-1, MICL, Dectin-2, Mincle/MCL, and the macrophage mannose receptor.

Received: 20 May 2016, Accepted: 1 August 2016, Published: 23 September 2016
Editor: Siamon Gordon, Oxford University, Oxford, United Kingdom
Correspondence: Gordon D. Brown, gordon.brown@abdn.ac.uk; Paul R. Crocker, p.r.crocker@dundee.ac.uk
© 2016 American Society for Microbiology. All rights reserved.
that recognizes sialylated glycans, followed by variable numbers of C2 set domains. Recognition of sialic acid depends on a conserved structural template involving both hydrogen bonding networks, ionic and hydrophobic interactions, together with variable interstrand loops that make contact with additional glycan residues and confer extended specificity to Siglecs (5). A Siglec-like Ig fold was recently seen in the regulatory myeloid receptor PILR (paired immunoglobulin-like type 2 receptor)-α and -β, which mediate high-affinity binding to mucin-like sialylated ligands via both protein-protein and protein-sialic acid interactions (6, 7). Siglecs are expressed broadly across the hematopoietic and immune systems, except for MAG, which is restricted to the myelin-forming cells of the nervous system, oligodendrocytes, and Schwann cells (Fig. 1). As discussed below, some Siglecs are highly restricted to particular cell types, whereas others are more broadly expressed. In humans and mice, the major subgroup of cells lacking Siglecs are CD4 and CD8 T cells, although in other species such as chimpanzees, Siglec-5 is reported to be expressed on all circulating T cells (8).

When expressed naturally at the cell surface, Siglecs interact with sialic acid-containing ligands both in cis (on the same plasma membrane) and in trans (on the plasma membrane of different cells). The degree to which each occurs likely depends on the relative affinity, density, and display of sialylated ligands as well as other poorly understood topographical constraints. Both types of interaction have been shown to play important roles in immune modulation (reviewed in references 9 and 10). The cytoplasmic tails of most Siglecs contain two or more tyrosine-based motifs that can be phosphorylated and recruit SH2 domain-containing effector molecules. The most common motif is the immunoreceptor tyrosine-based inhibitory motif (ITIM) that has been identified in hundreds of receptors of the immune system (11). Phosphorylated ITIMs exhibit high affinity for the tandem SH2 domain-containing protein tyrosine phosphatases SHP-1 and SHP-2, which are activated on binding to ITIMs and thereby potentially capable of modulating signaling functions of Siglec-expressing cells (9). Many Siglecs also possess ITIM-like motifs that appear to synergize with the ITIMs for efficient recruitment of SHP-1 and SHP-2 (12, 13). The same motifs are also important for the endocytic functions of many Siglecs. Some Siglecs possess a basic residue in the transmembrane region (Fig. 1) that leads to formation of a membrane complex with DNAX activation protein of 12 kDa (DAP12), an adaptor with an immunoreceptor tyrosine-based activation motif (ITAM). As a consequence, these Siglecs have the potential to directly trigger signal transduction via Syk recruitment and activation. Links between glycan recognition by Siglecs and subsequent intracellular signaling have been a major focus for many laboratories since their discovery, but the specific downstream targets and biochemical pathways remain elusive for the most part. The focus of this section will be Siglecs that are expressed predominantly on human myeloid cells, namely Sn (Siglec-1), CD33 (Siglec-3), and Siglec-5, -7, -8, -9, -10, -11, -14, and -15. Given the importance of mouse models in determining the biological functions of Siglecs, the murine CD33-related Siglec-E, -F, and -G will be grouped with their most closely related human counterparts for comparative purposes.

**Sialoadhesin (Siglec-1; CD169)**

Sn is a prototypic Siglec that was identified as a sialic acid-dependent erythrocyte receptor expressed by subsets of mouse tissue-resident macrophages (14). Sn has an unusually large number of 17 Ig domains that appear conserved in mammals and reptiles (15). These are important for extending the sialic acid-binding site away from the plasma membrane to promote intercellular interactions. Sn prefers α2,3-linked sialic acids over α2,6- and α2,8-linked sialic acids and does not bind sialic acids modified by hydroxylation (Neu5Gc) or 9-O-acetylation (e.g., Neu5Ac2) (16, 17).

In humans and mice, Sn expression appears specific for tissue macrophage subsets described as “CD169+ macrophages” (18–20). These cells are abundant in lymphoid tissues, notably subcapsular sinus macrophages in lymph nodes and marginal metallophilic

---

**TABLE 1 Lectin families**

<table>
<thead>
<tr>
<th>Family name</th>
<th>Selected ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calnexin</td>
<td>Glc₃Man₉</td>
</tr>
<tr>
<td>Chitinase-like lectins</td>
<td>GlcN, GalN, chitin, chito-oligosaccharides</td>
</tr>
<tr>
<td>C-type lectins</td>
<td>Various (e.g., mannose, fucose, GalNAc, β-glucan)</td>
</tr>
<tr>
<td>F-box lectins</td>
<td>High-mannose and sulfated glycoproteins</td>
</tr>
<tr>
<td>Ficolins</td>
<td>GlcNAc, GalNAc, fucose</td>
</tr>
<tr>
<td>F-type lectins</td>
<td>Fucose and others (e.g., 3-O-methyl-, β-galactose)</td>
</tr>
<tr>
<td>Galectins</td>
<td>β-Galactosides (e.g., N-acetyllactosamine)</td>
</tr>
<tr>
<td>Intelectins</td>
<td>Galactofuranose, pentoses</td>
</tr>
<tr>
<td>L-type lectins</td>
<td>Various (e.g., oligomannose)</td>
</tr>
<tr>
<td>M-type lectins</td>
<td>High-mannose glycans (e.g., Man₉GlcNAc₂)</td>
</tr>
<tr>
<td>P-type lectins</td>
<td>Mannose 6-phosphate</td>
</tr>
<tr>
<td>R-type lectins</td>
<td>Various (e.g., GalNAc, sialic acid, sulfated glycans)</td>
</tr>
<tr>
<td>Siglecs</td>
<td>Sialic acid</td>
</tr>
</tbody>
</table>

---

**References**

[10] C-type lectins Various (e.g., mannose, fucose, GalNAc, β-glucan)
macrophages in the spleens of rodents or perifollicular capillary sheaths in spleens of humans (21). These macrophage populations are strategically positioned to capture viruses and immune complexes from the afferent lymphatics and splenic sinuses, respectively, and may therefore share similar biological functions (22). Emerging evidence clearly shows that CD169+ macrophages play a key role in the capture of a broad range of viruses, including arteriviruses (23), retroviruses (24), herpes-viruses (25, 26), and adenoviruses (27), and for the first two, this directly involves viral recognition by Sn. CD169+ macrophage capture of viruses is important for restraining viral spread to distal sites (28), but it can also promote viral transfer to neighboring cells such as T cells (29, 30) and B cells (31) and directly prime CD8+ cytotoxic T cells for responses to viral antigens via cross-presentation (27). Interestingly, CD169+ macrophages in the spleen have been shown to act as “Trojan horses” for vesicular stomatitis viruses, permitting high viral replication that is important for stimulation of protective adaptive immune responses (32).

In addition to viruses, CD169+ macrophages can capture apoptotic tumor cells and cross-present tumor antigens to drive antitumor cytotoxic CD8 T-cell

**FIGURE 1.** Sigles in humans and mice. There are two subgroups of Sigles: One group contains Sigles that are conserved in all mammalian species and the other group contains CD33-related Sigles that appear to be undergoing rapid evolution in primates. The cell types expressing highest levels of each Siglec are indicated. B, B cell; Eos, eosinophil; Mac, macrophage; mDC, myeloid dendritic cell; Mon, monocyte; Neu, neutrophil; NK, NK cell; Oli, oligodendrocyte; Ost, osteoclast; pDC, plasmacytoid dendritic cell; Pla, placental syncytiotrophoblast; Sch, Schwann cell.
responses (33). Conversely, uptake of apoptotic cells by CD169+ macrophages can drive tolerance of self-reactive T cells via induction of the chemokine CCL22 (34). CD169+ macrophages can also transfer exogenous antigens to dendritic cells (DCs) and promote cross-presentation to CD8 T cells (35) and transfer antigens to B cells (36) and NKT cells (37) to promote cellular activation. Besides its constitutive high expression on tissue macrophage subsets, Sn can also be induced strongly on monocytes, macrophages, and monocyte-derived DCs in vitro by type I interferons or agents such as viruses and Toll-like receptor (TLR) ligands that induce interferon production (24). Accordingly, Sn is upregulated on circulating monocytes in HIV-infected individuals and on macrophages in rheumatoid arthritis (RA) (38), primary biliary cirrhosis (39), systemic sclerosis (40), and systemic lupus erythematosus (41). Sn expression on inflammatory macrophages has been associated with favorable prognosis in colorectal cancer (42) and in endometrial carcinoma (43), but with a more severe disease in proliferative glomerulonephritis (44). Many of the above disease associations may reflect exposure of macrophages to interferons rather than being causally related. Indeed, in the BWF1 murine model of spontaneous systemic lupus erythematosus, there was no influence of Sn deficiency on disease severity (45). However, in mouse models of inherited neuropathy (46–48), autoimmune uveoretinitis (49), and experimental allergic encephalomyelitis (50), Sn-deficient mice exhibited reduced inflammation accompanied by reduced levels of T-cell and macrophage activation. In the experimental allergic encephalomyelitis model, this appears to be due to an Sn-dependent suppression of CD4+FoxP3+ regulatory T-cell (Treg) expansion, thereby promoting inflammation (50), whereas in the other central nervous system models, Sn-dependent repression of CD8 T cells is important (46). The upregulation of sialylated ligands for Sn on activated T-cell populations is likely to be an important determinant in mediating the Sn-dependent suppression of T-cell subsets and function (51). Sn can also efficiently mediate the capture and uptake of exosomes released from B lymphocytes following apoptosis and therefore play a role in antigen presentation to T cells (52, 53).

A role for Sn in phagocytic interactions of macrophages with various sialylated bacterial and protozoal pathogens was initially demonstrated, including Neisseria meningitidis (54), Campylobacter jejuni (55), and Trypanosoma cruzi (56). Sn-dependent targeting of heat-killed C. jejuni to splenic red pulp macrophages led to a rapid induction of type I interferon and pro-inflammatory cytokines, in a MyD88-dependent manner, suggesting a host protective role for Sn against sialylated bacteria (57). This was also supported in an infection model using a sialylated strain of group B streptococcus (GBS), where Sn-deficient mice exhibited reduced bacterial spread (58). However, this protective role for Sn was only seen in neutrophil-depleted mice, suggesting that Sn-dependent macrophage bacterial uptake can provide a backup defense in the event of neutrophils failing to clear the bacteria. Conversely, Sn expression on macrophages and monocyte-derived DCs can be exploited by enveloped viruses displaying host-derived sialic acids, leading to their capture, uptake, and dissemination. This was first seen with the porcine reproductively and respiratory syndrome virus, which targets lung alveolar macrophages of pigs (23), and more recently with HIV (24) and other retroviruses (31, 59). On HIV, Sn can recognize both gp120, a sialylated glycoprotein, and GM3, a monosialylated ganglioside terminating in NeuAcα2-3Gal (30, 60–62). GM3 is packaged into the HIV envelope during the budding from infected cells that occurs in lipid rafts (63). On monocyte-derived DCs, Sn interactions with HIV lead to membrane invaginations containing viral particles that are very efficiently transferred to T cells in a process known as trans-infection (30). In vivo evidence that Sn promotes retroviral trans-infection was obtained following infection of mice using murine leukemia virus, where trans-infection of B cells depended on the expression of Sn on lymph node sinus-lining macrophages (31).

Although Sn is unusual among Siglecs in not having well-defined signaling motifs in its cytoplasmic tail, recent reports have suggested it can associate with the ITAM adaptor DAP12 and either suppress type I interferon production or stimulate transforming growth factor β production (64, 63). These studies were both done using RNA knockdown approaches to suppress Sn expression, and the physiological significance of the findings should be confirmed using primary macrophages from Sn-deficient mice.

**CD33 (Siglec-3)**

CD33 is a marker of early human myeloid progenitors and leukemic cells, and is also expressed on monocytes, tissue macrophages, NK-cell subsets, and weakly on neutrophils. It has two Ig domains and was the first of the CD33-related Siglecs to be characterized as an inhibitory receptor, suppressing activation of FcγRI and recruiting SHP-1 and SHP-2 (66). CD33 has some preference for α2,6- over α2,3-sialylated glycans and binds
strongly to sialylated ligands on myeloid leukemic cell lines (67). The restricted expression of CD33 has been exploited in the treatment of acute myeloid leukemia using gemtuzumab, a humanized anti-CD33 monoclonal antibody (mAb) coupled to the toxic antibiotic calicheamicin. Binding of anti-CD33 mAbs to CD33 triggers endocytosis of the bound antibody. This depends on ITIM phosphorylation, recruitment of the E3 ligase Cbl, and ubiquitylation of the CD33 cytoplasmic tail (68–70). Selective expression of CD33 on leukemic progenitor cells also makes it an attractive target for therapy using chimeric antigen receptors expressed on cytotoxic T cells.

Recently, two coinherited single-nucleotide polymorphisms (SNPs) have been associated with protection of humans against late-onset Alzheimer’s disease in genome-wide association studies. These SNPs result in increased exon 2 skipping, leading to raised levels of CD33 lacking the V-set domain and reduced levels of full-length CD33 (71). Since full-length CD33 can inhibit microglial cell uptake of amyloid-β protein in a sialic acid-dependent manner (72, 73), it is thought that individuals lacking the protective SNPs may accumulate more toxic amyloid-β proteins, thus driving pathology. Targeting CD33 using antibodies that either inhibit function or promote internalization and degradation may be a useful approach to treating Alzheimer’s disease.

The murine ortholog of CD33 exists as two spliced forms that differ in the cytoplasmic tail, neither containing the typical ITIM found in most other CD33-related Siglecs (74). Furthermore, mCD33 has a lysine residue in the transmembrane sequence and may therefore couple to the DAP12 transmembrane adaptor, as shown for mouse Siglec-H (75) and human Siglec-14 (76), -15 (77), and -16 (78). In contrast to hCD33, mCD33 in the blood is expressed mainly on neutrophils rather than monocytes, which also suggests a non-conserved function of this receptor (79).

Siglec-5 (CD170) and Siglec-14

The SIGLEC5 and SIGLEC14 genes are adjacent to each other on chromosome 19 and encode proteins containing four and three Ig-like domains, respectively. The first two Ig domains of Siglec-5 and -14 share >99% sequence identity but then diverge. Siglec-5 is an inhibitory receptor with typical ITIMs, whereas Siglec-14 is complexed with DAP12 and mediates activatory signaling. Both Siglec-5 and -14 bind similar ligands, with a preference for the sialyl-Tn structure ( Neu5Acα2-6GalNAcα ) (76). Although many antibodies to Siglec-5 cross-react with Siglec-14, specific antibodies have shown that while Siglec-5 is expressed on neutrophils and B cells, Siglec-14 is found at low levels on neutrophils and monocytes. A SIGLEC14 null allele is frequently present in Asian populations but is less common in Europeans (80). This is due to a recombination event between the S’ region of the SIGLEC14 gene and the 3′ region of the SIGLEC5 gene, resulting in a fusion protein that is identical to Siglec-5 but expressed in a Siglec-14-like manner. Individuals with chronic obstructive pulmonary disease who are SIGLEC14 null exhibited reduced exacerbation attacks compared with individuals expressing Siglec-14 (80). Siglec-5 can bind sialylated strains of N. meningitidis, and both Siglec-5 and -14 can bind sialylated strains of Haemophilus influenzae implicated in chronic obstructive pulmonary disease exacerbations and trigger inhibitory and activatory responses, respectively (81). Thus, the absence of Siglec-14 on neutrophils would lead to reduced inflammatory responses in SIGLEC14-null individuals. Besides expression on leukocytes, both Siglec-5 and -14 are found on human amniotic epithelium and may influence responses to GBS infection and the frequency of preterm births in infected mothers (81). Besides mediating sialic acid-dependent interactions with host cells and pathogens, Siglec-5 and -14 can mediate sugar-independent interactions with some strains of GBS via recognition of the β protein (81). A recent study also demonstrated that the nonglycosylated danger-associated molecular pattern (DAMP) protein heat shock protein 70 (Hsp70) can bind to Siglec-5 and -14 and modulate cellular responses (82). There are no obvious equivalents of Siglec-5 or -14 in mice, making it difficult to study this interesting pair of receptors in animal models.

Siglec-7, -9, and -E

Siglec-7 and -9 share a high degree of sequence similarity and appear to have evolved by gene duplication from an ancestral gene encoding a three-Ig-domain inhibitory Siglec, represented in mice by Siglec-E. Siglec-7 is the major Siglec on human NK cells and is also seen at lower levels on monocytes, macrophages, DCs, and a minor subset of CD8 T cells (83–85). Siglec-7 has also been detected in platelets, basophils, and mast cells, where it may modulate survival and activation (85). Siglec-9 is prominently expressed on neutrophils, monocytes, macrophages, and DCs; ~30% of NK cells; and minor subsets of CD4 and CD8 T cells (86, 87). Despite high sequence similarity, Siglec-7 binds strongly to α2,8-linked sialic acids present in “b-series” gangliosides (and some glycoproteins, whereas Siglec-9 prefers α2,3-linked sialic acids) (88). Sulfation of the sialyl Lewis X (sLeX)
structure can strongly influence recognition by both Siglecs, with Siglec-9 preferring 6-sulfo-sLeX and Siglec-7 binding well to both 6-sulfo-sLeX and 6′-sulfo-sLeX (89). It has recently been shown that Siglec-9 can bind strongly to high-molecular-weight hyaluronan, and that its ligation on neutrophils leads to suppression of cellular activation (90). Siglec-E in mice exhibits a combination of some features of Siglec-7 and Siglec-9, being mainly expressed on neutrophils, monocytes, and macrophages, with sialic acid-binding preferences that span those of both Siglec-7 and -9 (91). Similar to T cells, NK cells in mice appear to lack expression of inhibitory Siglecs. Siglec-E is an important inhibitory receptor of neutrophils, as initially demonstrated in a lipopolysaccharide (LPS)-induced lung inflammation model in which Siglec-E-deficient mice exhibited exaggerated CD11b-dependent neutrophil influx (92). This was found to be linked to Siglec-E-dependent production of reactive oxygen species (ROS) by neutrophils triggered on the CD11b ligand fibrinogen, which suppressed neutrophil recruitment to the lung (93). Siglec-E-dependent inhibition of neutrophil function has also been proposed to be a mechanism underlying an exaggerated aging phenotype observed in one strain of Siglec-E-deficient mice (94). Several studies have also demonstrated inhibitory functions of Siglec-E on macrophages and DCs, including suppression of proinflammatory cytokine production in response to TLR ligands and promotion of Tregs in response to sialylated antigens (95–99). Furthermore, targeting Siglec-E on macrophages with sialylated nanoparticles was shown to block inflammatory responses in vitro and in vivo (100).

Tumor cells often upregulate cell surface sialylated glycans, and it appears that these may be important in Siglec-dependent dampening of antitumor immunity. Siglec-7 and -9 can both suppress NK-cell cytotoxicity against tumor cells expressing relevant glycan ligands (101–103). Siglec-9 and Siglec-E can also dampen neutrophil activation and tumor-cell killing, while ligation of Siglec-9 or Siglec-E on macrophages by tumor glycans seems to suppress formation of tumor-promoting M2 macrophages (98). Studies with GBS have also demonstrated that sialylated bacteria can subvert innate immune responses by targeting Siglec-9 and Siglec-E on neutrophils and macrophages, resulting in attenuation of phagocytosis, killing, and proinflammatory cytokine production (104, 105).

**Siglec-8 and -F**

Siglec-8 has three Ig domains and is expressed on eosinophils and mast cells, with weaker expression on basophils (106, 107). It binds strongly to 6′-sulfo-sLeX and to mucins isolated from bronchial tissues (108, 109), but endogenous mucin ligands do not seem to require sulfation for strong binding (110). In mast cells, antibodies to Siglec-8 can inhibit FcεRI-triggered degranulation responses, in line with its role as an inhibitory receptor (111). In eosinophils, much attention has focused on the role of Siglec-8 in triggering apoptosis, which can occur following cross-linking with anti-Siglec-8 antibodies or sialoglycan polymers (112, 113). Apoptosis depends on generation of ROS and caspase activation and is paradoxically enhanced in the presence of cytokine “survival” factors such as granulocyte-macrophage colony-stimulating factor and interleukin-5 (IL-5) (113). A role for Siglec-8 in the pathogenesis of asthma has been suggested by upregulation of Siglec-8 ligands in inflamed lung tissue (109) and by associations of Siglec-8 polymorphisms with asthma (114).

Although there is no ortholog of Siglec-8 in mice, the four-Ig-domain mouse Siglec-F is expressed in a similar way to Siglec-8 on eosinophils, has a similar glycan-binding preference for to 6′-sulfo-sLeX, and appears to have acquired similar functions through convergent evolution (115–117). There are some important differences, however. Siglec-F can recognize a broader range of α2,3-linked sialic acids; it is also expressed on alveolar macrophages and triggers weaker apoptosis using different signaling pathways (118). Siglec-F-null mice show exaggerated eosinophilic responses in certain lung allergy models, suggesting that Siglec-F negatively regulates eosinophil production and/or survival following immunological challenge (119, 120). Interestingly, Siglec-F ligands in the airways and lung parenchyma were also upregulated during allergic inflammation, but these did not appear to require sulfation to mediate strong binding to Siglec-F (110).

**Siglec-10 and -G**

Siglec-10 has five Ig-like domains and, in addition to the ITIM and ITIM-like motifs, displays an additional tyrosine-based motif in its cytoplasmic tail (121–123). It is expressed at relatively low levels on several cells of the immune system, including B cells, monocytes, and eosinophils (122). It can also be strongly upregulated on tumor-infiltrating NK cells in hepatocellular carcinoma, where its expression was negatively associated with patient survival (124). It is the only CD33-related human Siglec that has a clear-cut ortholog in mice, designated Siglec-G (9). Both Siglec-10 and Siglec-G prefer Neu5Gc over Neu5Ac in both α2,3 and α2,6 linkages (125). Similar to Siglec-10 in humans and pigs (126), Siglec-G...
is expressed mainly on B cells and subsets of DCs and weakly on eosinophils (127, 128). Mice deficient in Siglec-G show a 10-fold increase in numbers of a specialized subset of B lymphocytes, the B1a cells, which make natural antibodies (129). These Siglec-G-deficient B1a cells also show exaggerated Ca fluxing following B-cell receptor cross-linking. Studies using knock-in mice carrying an inactivating mutation in the sialic acid-binding site of Siglec-G show a similar phenotype (127). This appears to be due to a requirement of sialic acid-dependent cis-interactions between Siglec-G and the B-cell receptor. On DCs, Siglec-G has been proposed to regulate cytokine responses to DAMPs released by necrotic cells in sterile inflammation. This is thought to be due to a dampening effect of cis-interactions between Siglec-G and the heavily sialylated DAMP receptor CD24 (130). Disruption of this interaction through sialidases released by bacteria such as Streptococcus pneumoniae may be important in triggering inflammatory responses in sepsis (131). A recent study has also shown that pseudaminic acid expressed on the flagella of C. jejuni can be recognized by Siglec-10 and trigger IL-10 production in DCs (132). This suggests a novel form of glycan recognition by Siglec-10 that is exploited by some pathogens.

**Siglec-11 and -16**

Siglec-11 and -16 are paired inhibitory and activatory receptors, with five and four Ig domains, respectively (78, 133). In most humans, the SIGLEC16 gene has a 4-bp deletion and only ~35% of humans express one or two functional alleles. The extracellular regions of these proteins are >99% identical due to gene conversion events, and anti-Siglec-11 mAb 4C4 cross-reacts with Siglec-16. Siglec-11 binds weakly to α2,8-linked sialic acids in vitro. Siglec-11 appears to be absent from circulating leukocytes but is expressed widely on populations of tissue macrophages, including resident microglia in the brain, where high levels of α2,8-linked sialic acids are present on gangliosides. Expression of Siglec-11 on microglia can impair their phagocytosis of apoptotic cells and neurotoxicity (134). Polysialic acid presented by neural cell adhesion molecule is also α2,8 linked and was shown to be recognized by Siglec-11 on macrophages and suppress LPS-dependent tumor necrosis factor α (TNF-α) production and phagocytosis triggered by LPS exposure (134, 135). Interestingly, microglial expression appears to be unique to humans (136). In mice, its function may be mediated by Siglec-E, which is similarly expressed on microglia and able to mediate neuroprotective effects in response to inflammatory signals (96). The activating receptor Siglec-16 is also present on macrophages, including those in the brain, but functional studies have not been reported (78).

**Siglec-15**

Siglec-15 was first described in 2007 as a highly conserved and ancient Siglec found in vertebrates (77). It lacks the typical arrangement of cysteines seen in the V-set Ig domain of other Siglecs and has an unusual intron-exon arrangement. Nevertheless, it can bind the sialyl-Tn structure (Neu5Acα2-6GalNAcα), with weaker binding to 3′-sialyllactose. It is associated with DAP12 and also has a tyrosine-based motif in the cytoplasmic tail. On macrophages, interactions with sialyl-Tn antigens expressed by tumor cells were shown to trigger transforming growth factor β production, which could be important in immunosuppression and promoting tumor growth (137).

Although it was first reported as being expressed on macrophages and DCs in human lymphoid tissues, subsequent work has established that Siglec-15 is most strongly expressed in osteoclasts and their precursors, where it plays an important role together with receptor activator of NF-κB ligand (RANKL) in triggering osteoclast differentiation (138–141). Osteoclasts are key cells involved in bone degradation and share a common hematopoietic progenitor with macrophages. Mice lacking Siglec-15 show a mild osteopetrosis and impaired osteoclast differentiation (140, 141). Specific antibodies directed to Siglec-15 are able to phenocopy this due to antibody-induced internalization and degradation of Siglec-15 (142). Siglec-15 therefore provides a novel target for diseases involving excessive osteoclast activation and bone loss, such as menopause-related osteoporosis.

**C-TYPE LECTIN RECEPTORS**

C-type lectin receptors (CLRs) are a diverse collection of >1,000 proteins and are the largest lectin family (143). All of these receptors possess at least one C-type lectin-like domain (CTLD), a characteristic fold formed by disulfide linkages between highly conserved cysteine residues (143). Based on their phylogeny and structure, CLRs have been divided into 17 groups that are either membrane bound or secreted (143). The term “C-type lectin” originated from initial observations that these receptors required Ca2+ for carbohydrate recognition. However, we now know that not all CLRs require Ca2+ for ligand recognition and that these receptors can recognize a much more diverse range of ligands, such as lipids and proteins, for example. Many of these receptors have also been shown to bind to different
classes of ligands (i.e., they are multivalent) and can recognize both endogenous and exogenous ligands.

A great many CLRs have essential roles in immunity. A key example is the endothelial-expressed selectins that function as adhesion molecules by binding cell surface glycoproteins on leukocytes and play a critical part in leukocyte migration during inflammation (144). Other examples include the secreted collectins, which function in both pulmonary physiology and immunity, and serum mannose-binding protein (MBL), which has an essential role in triggering complement activation through MBL-associated serine proteases in response to microbial infection (145). The focus of the rest of this section, however, will be on selected transmembrane receptors that are widely expressed by myeloid cells and have been extensively characterized in murine models, including Dectin-1, MCL (myeloid inhibitory C-type lectin), Dectin-2, Mincle (macrophage inducible C-type lectin receptor)/MCL (macrophage C-type lectin), and the macrophage mannose receptor (MR). Detailed descriptions of these molecules will serve as illustrative examples of the varied nature of C-type lectins and their importance in myeloid cell function in health and disease. The functions and properties of other myeloid-expressed CLRs, including well-characterized receptors such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), can be found in several excellent reviews (146–148).

**Dectin-1 (CLEC-7A)**

Dectin-1 is one of the best-characterized myeloid-expressed CLRs, and this type II transmembrane receptor belongs to group V within the CLR family. Dectin-1 contains a single extracellular CTLD, a stalk region, a single-pass transmembrane domain, and a cytoplasmic tail containing signaling motifs, including an ITAM-like (or hem-ITAM) motif and a triacidic motif (Fig. 2). Dectin-1 is alternatively spliced into two major isoforms, differing by the presence or absence of the stalk region, which are expressed differentially in different cell types and mouse strains and which have slightly different functionalities (149). The receptor is N-glycosylated, which can affect its expression and function (150), and is predominantly expressed by myeloid cells, including monocytes, macrophages, DCs, and neutrophils (151). There is also evidence for expression of this receptor on B cells and subsets of T cells, and it may be unregulated on epithelial cells during inflammation (151–154).

Through mechanisms that are not yet completely understood, the CTLD of Dectin-1 is able to recognize β-1,3-glucan-containing carbohydrates (155). These carbohydrates are found predominantly in fungal cell walls, and consequently there has been considerable focus on the role of Dectin-1 in antifungal immunity. Indeed, Dectin-1 recognizes many fungal species, including major human pathogens such as *Aspergillus, Candida, Coccidioides*, and *Pneumocystis* (156). There is now substantial evidence that Dectin-1 plays an essential role in antifungal immunity: several polymorphisms of this receptor in humans (including a Y238X polymorphism that essentially renders homozygous individuals Dectin-1 deficient) have been linked to increased susceptibility to mucocutaneous fungal infections or fungal-induced inflammation in the gut (157–159). Moreover, Dectin-1 knockout mice are more susceptible to systemic and mucocutaneous infections with several pathogens (160–162). However, the requirement for Dectin-1 for controlling *Candida albicans in vivo* is dependent on the fungal strains, which undergo differential changes in their cell wall during infection (163).

In addition to fungi, Dectin-1 can recognize mycobacteria. How Dectin-1 recognizes these pathogens is unknown, and although shown to promote IL-12 responses in vitro, the receptor does not appear to play an essential role in antimycobacterial immunity in vivo (164, 165). Dectin-1 has also been implicated in the recognition of other pathogens, including *Leishmania* (166).

Dectin-1 was originally identified as acting as a T-cell costimulatory molecule through recognition of an endogenous ligand (167), but the nature of this ligand remains elusive. Several other endogenous ligands have been described, including vimentin, through which Dectin-1 was thought to be involved in driving lipid oxidation in atherosclerosis (168). However, Dectin-1 deficiency was subsequently found not to affect atherosclerosis development in mouse models (169). Dectin-1 has also been implicated in the reverse transcytosis of secretory IgA-antigen complexes by intestinal M cells and induction of subsequent mucosal and systemic antibody responses (170). Moreover, in the presence of galactosylated IgG1, Dectin-1 associates with FcγRIIB, resulting in the inhibition of complement-mediated inflammation (171). In response to intestinal mucus, FcγRIIB, along with another lectin, galectin-3, complexes with Dectin-1 to promote the anti-inflammatory properties of DCs, enhancing homeostasis and oral tolerance (172). Most recently, a protective role for Dectin-1 in antitumor immunity has been demonstrated. Mechanistically, Dectin-1-mediated recognition of N-glycan structures on tumor cells was shown to augment NK-mediated killing and, in a model of...
hepatocarcinogenesis, act protectively by suppressing TLR4 signaling (173, 174).

Upon recognition of β-glucans, Dectin-1 can activate Syk-dependent and Syk-independent intracellular signaling cascades. Surprisingly, the activation of Syk was shown to require the tyrosine phosphatase SHP-2, which acted as a scaffold and facilitated the recruitment of Syk to Dectin-1 (175). The ability of Dectin-1 to induce Syk-dependent signaling pathways is mediated by a single phosphorylated tyrosine residue in the ITAM-like motif within the cytoplasmic tail and is likely to require receptor dimerization (176). Signaling through this pathway involves protein kinase C-δ (PKC-δ) and the caspase recruitment domain family member 9 (CARD9)-Bcl10-Malt1 complex and leads to the induction of canonical and noncanonical NF-κB subunits and interferon regulatory factor 1 (IRF1), resulting in gene transcription (177, 178). Recently, CARD9 was found to be dispensable for NF-κB activation, but regulated extracellular signal-regulated kinase (ERK) activation by linking Ras-GRF1 to H-Ras (179). The CARD9 pathway is utilized by several other receptors (see also below).
and is essential for protective antimicrobial immunity, particularly against fungi (180–182). Syk activation by Dectin-1 induces IRF5 and nuclear factor of activated T cells (NFAT), through phospholipase C-γ (PLC-γ) and calcineurin (183, 184), a pathway inhibited by immunosuppressive drugs, such as cyclosporine, and linked to the increased susceptibility to fungal infection that occurs following administration of these compounds (185). The Syk-independent pathway from Dectin-1 involves activation of Raf-1, which integrates with the Syk-dependent pathway at the point of NF-κB activation (186). Other pathways also exist. For example, the induction of phagocytosis by Dectin-1 in macrophages is Syk independent, requiring Bruton’s tyrosine kinase (Btk) and Vav-1 (187, 188). The ability of Dectin-1 to induce productive intracellular signaling (i.e., leading to cellular responses) requires receptor clustering into a “phagocytic synapse” and exclusion of regulatory tyrosine phosphatases (189). Moreover, the ability of Dectin-1 to induce productive responses to purified agonists can be cell type specific, an effect linked to differential utilization of CARD9 (190).

Activation of Dectin-1 signaling pathways can induce multiple cellular responses, including actin-mediated phagocytosis (Fig. 3), phagosome maturation, activation of the respiratory burst, regulation of neutrophil extracellular trap (NET) formation in neutrophils, DC maturation, and antigen presentation, in part through the use of autophagy machinery (191–194). Dectin-1 can activate inflammasomes, facilitating the production of IL-1β. Indeed, this receptor has been implicated in activation of the Nlrp3 (NLR family, pyrin domain-containing 3) inflammasome, although the pathways involved are unclear, and can directly induce the noncanonical caspase-8 inflammasome, through CARD9 and Malt1 (195–197). Assembly and activation of the caspase-8 inflammasome was recently shown to require the nonreceptor tyrosine kinase Tec (198). Dectin-1 also induces the production of eicosanoids and several cytokines and chemokines (including TNF, IL-10, IL-6, IL-23, CCL2, and CCL3), and can modulate cytokine production and cellular functions induced by other pattern recognition receptors. For example, costimulation of Dectin-1 and MyD88-coupled TLRs leads to the synergistic production of cytokines, such as TNF and IL-23, while simultaneously repressing the induction of others, such as IL-12 (199, 200). Another example is the ability of Dectin-1 to activate complement receptor 3 (CR3; alternatively Mac-1), through activation of Vav1, Vav3, and PLC-γ, which results in enhanced neutrophil phagocytosis and ROS production (201). These two receptors also act collaboratively in macrophages, through association in lipid rafts and activation of the Syk-Jun N-terminal kinase–activator protein 1 (AP1) pathway, to enhance inflammatory cytokine responses (202).

Like the TLRs, Dectin-1 is capable of instructing the development of adaptive immune responses, particularly Th1 and Th17 immunity (203). Interestingly, Dectin-1-activated DCs can also instruct Tregs (CD25+Foxp3+) to express IL-17 (204). While Th1 responses are important for the control of systemic infections, Th17 responses are critical for controlling fungal infections at the mucosa. Indeed, several human diseases associated with chronic mucocutaneous candidiasis, including CARD9 deficiency, have been linked to alterations in components of the Th17 response (205). How Dectin-1 promotes Th17 responses is incompletely understood, but involves Malt1-dependent activation of the NF-κB subunit c-Rel, which is required for the induction of polarizing cytokines such as IL-1β and IL-23p19 (206). Dectin-1 can also induce humoral responses (207), stimulate cytotoxic T-cell responses (208), and induce myeloid-derived suppressor cells, which can suppress T- and NK-cell responses (209). In addition to classic adaptive immunity, activation of Dectin-1 has been shown to induce innate immune memory (or trained immunity), through the epigenetic reprogramming of monocytes that occurs following aerobic glycolysis.
induced through an Akt–mammalian target of rapamycin (mTOR)–hypoxia-inducible factor-1α (HIF1α) pathway (210, 211).

The role of Dectin-1 in driving adaptive immunity during infection is still not completely understood, but there has been some recent progress. For example, Dectin-1 was found not to be essential for IL-17 production in mice systemically infected with *C. albicans* (203), yet was required to drive Th17 polarization during pulmonary infection with *Aspergillus fumigatus* (162, 212). The ability of Dectin-1 to induce T helper cell differentiation during a skin infection model with *C. albicans* was recently shown to be dependent on fungal morphology (correlating with β-glucan exposure) and the DC subset involved (213). In the gastrointestinal tract, Dectin-1 was found to be essential for driving fungal-specific CD4+ T-cell responses and for the maintenance of the cellularity of gastrointestinal-associated lymphoid tissues (214). Dectin-1 can also regulate intestinal Treg-cell differentiation through modification of the microbiota, following exposure to dietary β-glucans (215).

**MICL (CLEC-12A)**

MICL (also called DCAL-2, CLL-1, and KLRL-1) is structurally similar to Dectin-1 and located in the same genomic region (192). Unlike Dectin-1, MICL is one of the few myeloid-expressed CLRs that contains an ITIM in its cytoplasmic tail, and, like some of the Siglecs described above, can induce inhibitory intracellular signaling through SHP-1 and SHP-2 phosphates (216). Human MICL is alternatively spliced into at least three isoforms (α, β, and γ), and the receptor is expressed as a monomer and heavily glycosylated (216). These latter features differ in the murine ortholog, which is expressed as a dimer and is only moderately glycosylated (217). In both species, MICL is expressed primarily by myeloid cells, including macrophages, monocytes, DCs, and granulocytes, although the receptor is also expressed on B cells, CD8+ T cells, and bone marrow NK cells in the mouse (217, 218). Expression levels of MICL are substantially regulated during inflammatory processes both in vitro and in vivo (217, 218). Interestingly, MICL is highly expressed on acute myeloid leukemia cells, and the receptor has been put forward as a marker of this disease as well as for developing antibody-directed immunotherapies (219–222). In addition, murine MICL has been proposed to be a marker for a distinct subset of CD8α- DCs (223). In mouse, targeting of antigens to MICL was found to induce CD4 and CD8 T-cell proliferation and enhance antibody responses (224).

MICL functions as an inhibitory receptor, and experiments with receptor chimeras have directly demonstrated that MICL can inhibit the activation signals induced through other pattern recognition receptors (216). Moreover, antibody cross-linking experiments have shown that MICL can inhibit NK-cell cytotoxicity (225) and differentially modulate DC responses, such as IL-12 production, depending on the mode of activation (226). Recently, MICL was shown to be regulated in an ATG16L1 (autophagy-related protein 16-like 1)-dependent manner and play a key role in antibacterial autophagy through a functional interaction with an E3-ubiquitin ligase complex (227).

MICL recognizes an endogenous ligand in many tissues and was recently identified as a receptor for dead cells and uric acid (217, 228). MICL was shown to be required to suppress the inflammatory responses induced by these ligands (228). Similar observations have been made with human leukocytes (229). Thus, MICL appears to have an important role in controlling damage-induced inflammation and may be involved in autoimmune diseases. Indeed, MICL was recently found to play an essential role in regulating myeloid cell-mediated inflammation in a murine model of RA (230). Although polymorphisms of *CLEC12A* do not associate with RA, autoantibodies to MICL were identified in a subset of RA patients, which, in mouse models, could exacerbate the disease (230). These findings suggest that the threshold of myeloid cell activation can be modulated by autoantibodies that bind to these types of inhibitory receptors. Downregulation of this receptor has also been proposed to underlie hyperinflammatory responses observed in Behçet’s syndrome and gout (231).

**Dectin-2 (CLEC4n)**

Dectin-2 has a structure similar to that of Dectin-1, except that it possesses a short cytoplasmic tail lacking recognizable signaling motifs (232). To mediate intracellular signaling, this receptor associates with the ITAM-containing Fcγγ adaptor molecule (232). Dectin-2 is unusual in this respect in that its interaction with the adaptor is mediated by a membrane-proximal region within its intracellular tail rather than through a transmembrane arginine residue as occurs with other similarly structured receptors (233). As with Dectin-1, signaling from the ITAM motif following Dectin-2 ligation occurs through the Syk, PKC-δ, and CARD9-Bcl10-Malt1 pathway, but also involves PLC-γ2 (233–238). Dectin-2 is expressed primarily by myeloid cells, including macrophages, subsets of DCs, and neutrophils, as well as monocytes, where its expression can be...
markedly upregulated during inflammation (206, 239–241).

Dectin-2 recognizes high-mannose-based structures through its “classical” carbohydrate-binding CTLD, which possesses a conserved EPN motif (242). This ligand specificity enables recognition of a variety of pathogens (including bacteria and nematodes, for example) and pathogen-derived molecules (including house dust mite allergens) (232). Recently, Dectin-2 was shown to recognize mannose-capped lipoarabinomannan of mycobacteria and play a role in antimycobacterial immunity (243). However, most attention has focused on the role of Dectin-2 in antifungal immunity, where it is required for protection against infection with selected fungal species, including C. albicans (through recognition of α-mannans on specific morphological forms) and Candida glabrata (233–236, 244). Dectin-2 can also recognize species of Malassezia, through an O-linked mannosie-rich glycoprotein; Blastomyces dermatitidis; Cryptococcus neoformans; Fonsecaea pedrosii; and A. fumigatus (245–249). In addition to pathogens, Dectin-2 may recognize an endogenous ligand and be involved in modulating UV-induced immunosuppression (250).

Like Dectin-1, Dectin-2 induces several cellular responses in response to microbial stimuli and can influence the development of adaptive immunity. In response to C. albicans, for example, Dectin-2 was shown to drive inflammatory host cytokine responses, including TNF, IL-6, and IL-12, and the development of Th17 and Th1 immunity (233, 235, 236, 248). Notably, Dectin-2 was found to selectively induce Th17-polarizing cytokines, including IL-23 and IL-1β, by activating the NF-κB subunit, c-Rel, via Malt1 (206). More recently, Dectin-2 was found to regulate a key neutrophil IL-17 autocrine loop during fungal infection (247). This receptor also plays a role in the physical recognition of fungi, and signaling from Dectin-2 can induce Nlrp3 inflammasome activation, extracellular trap formation, the respiratory burst, and production of cysteiny1 leukotrienes (238, 241, 251–254). Dectin-2 may also form heterodimeric complexes with MCL (Dectin-3), although this is still controversial (255, 256).

The induction of cysteiny1 leukotrienes, in particular, has led to a great deal of interest in the role of Dectin-2 in airway inflammation induced by house dust mite. This CLR can recognize a glycan component of house dust mite, inducing the production of cysteiny1 leukotrienes by DCs and stimulating the development of Th2 responses (252, 257). In mouse models of house dust mite–mediated pulmonary inflammation, Dectin-2 drove eosinophilic and neutrophilic responses by promoting both Th17 and Th2 immunity (257–260). Despite a clear role for Dectin-2 in allergy and host defense in mouse models, there is only one report demonstrating a link between polymorphism in this receptor and human disease (pulmonary cryptococcosis) (261).

**Mincle/MCL (CLEC4d/CLEC4e)**

Mincle and MCL (also known as CLECSF8 or Dectin-3) are similar in structure to Dectin-2, but are discussed here together because they form a heterotrimeric complex (along with the ITAM-containing FcRγ adaptor) that is required for expression at the cell surface (256, 262–265). The FcRγ adaptor appears to associate primarily with Mincle, through a positively charged arginine residue in the transmembrane domain, and can induce signaling through the Syk, PKC-δ, CARD9-Bcl10-Malt1, and mitogen-activated protein kinase pathways, leading to activation of transcription factors, including NF-κB (237, 262, 266). This adaptor can also associate with MCL, but this occurs in an unusual fashion independently of any charged amino acid residue in the transmembrane or cytoplasmic domain (264, 267). The association of MCL with Mincle is mediated by the stalk region, and expression of these receptors is coordinately regulated under naive and inflammatory conditions (256, 265). The CTLD of MCL has also been shown to be involved in regulating surface expression (267).

Unsurprisingly, given that they function as a complex, there is significant overlap in the reports describing the expression and function of Mincle and MCL. Both of these receptors have been described as being predominantly expressed on myeloid cells, including macrophages, neutrophils, monocytes, and DCs, although there is also evidence of expression on other leukocytes, including some subsets of B cells (256, 262, 267–273). Expression of these receptors can be upregulated following exposure to inflammatory stimuli, including microbial components such as LPS, and for Mincle this has been shown to occur in a MyD88- and CCAAT enhancer-binding protein β (C/EBPβ)-dependent manner (256, 274, 275). Mincle has also been reported to be reciprocally expressed on neutrophils and monocytes within individuals, which has functional implications (269).

Mincle and MCL have both been shown to induce and/or regulate numerous cellular responses including endocytosis, phagocytosis, the respiratory burst, activation of the Nlrp3 inflammasome, NET formation, and the production of proinflammatory cytokines and
chemokines (TNF, macrophage inflammatory protein 2 [MIP-2], IL-1β, MIP-1α, IL-6, keratinocyte-derived chemokine, and granulocyte colony-stimulating factor, for example) (237, 255, 262–264, 267, 272, 276–279). Moreover, both receptors can modulate the development of adaptive immunity, promoting Th1 and Th17 responses (264, 277, 278). Mincle has also been shown to promote Th2 development by suppressing Dectin-1-mediated IL-12 production (178).

The CTLD of Mincle contains a classical mannose-recognition EPN motif, but the receptor appears to primarily recognize microbial glycolipids (280–283). Specific microbial ligands have been identified, including mycobacterial cord factor, trehalose dimycolate (TDM), its synthetic analog trehalose dibehenate (TDB), glycerol monomycolate from mycobacteria, and glyceroglycolipid and mannosyl-linked mannosyl fatty acids from fungi (245, 277, 284). Structural analysis suggests that Mincle’s CTLD has binding sites for both the sugar and fatty acid moieties of these ligands (282, 283). In contrast, the CTLD of MCL is unable to directly recognize carbohydrates (267), but this receptor can recognize TDM (264). Structural analysis has suggested that, like Mincle, the CTLD may interact with both the sugar and fatty acid moieties of this glycolipid (282).

Given the ability of these CLR to recognize mycobacterial components, it is not surprising that both receptors have been implicated in antimycobacterial immunity. In response to mycobacterial ligands, for example, Mincle induces the production of inflammatory cytokines and nitric oxide, granuloma formation, and Th1 and Th17 responses (271, 272, 277, 278, 285). These activities of Mincle contribute to the adjuvant activities of complete Freund’s adjuvant (286). MCL was similarly found to be required for the adjuvant activity of TDM, and loss of this receptor impaired both innate (inflammation and granuloma formation) and adaptive responses (T-cell function) induced by this glycolipid (264). Mincle has also been shown to recognize intact mycobacteria in vitro, but its actual role in vivo during infection is still unclear (277, 278, 287). One group has reported no effect of Mincle deficiency on infections with Mycobacterium tuberculosis H37Rv, whereas other groups have described some alterations in inflammation and bacterial burdens following infection with Mycobacterium bovis BCG or M. tuberculosis Erdman (271, 287, 288). In contrast, MCL was recently discovered to have an essential role in the nonopsonic recognition of mycobacteria by myeloid cells, and loss of this receptor resulted in higher extracellular mycobacterial burdens that drove neutrophilic inflammation and increased mortality in mouse models (289). Importantly, a polymorphism of MCL was also shown to be associated with susceptibility to tuberculosis in humans (289).

Both Mincle and MCL can also recognize other bacteria, including Klebsiella pneumoniae. During K. pneumoniae infection, for example, MCL−/− mice showed increased susceptibility and presented with increased bacterial burdens, inflammatory neutrophilic responses, and severe lung pathology (290). Mincle has similarly been found to be required for the control of K. pneumoniae infection in mouse models (279).

Mincle was first characterized as a receptor for C. albicans (291), and in response to this fungal pathogen, Mincle can induce protective immune responses including phagocytosis, fungal killing, and inflammatory cytokine production (269, 291). As mentioned above, Mincle was found to be reciprocally expressed on leukocytes within the same individuals, and in monocytes expression correlated with reduced fungal uptake and killing, but enhanced inflammatory cytokine production. In contrast, expression of Mincle on neutrophils correlated with enhanced fungal uptake and killing (269). MCL−/− mice were also shown to display increased susceptibility to C. albicans, with higher fungal burdens, and defective inflammatory responses (255). However, these observations are controversial, as other groups have not found any evidence for a role of MCL in anti-Candida immunity (263, 267, 289).

Mincle has been implicated in immunity to Malassezia and was found to be required for cytokine induction and inflammation during in vivo infection (280). In addition, Mincle recognizes F. pedrosoi and Fonsecaea monophora, causative agents of chromoblastomycosis (178, 292). In contrast to other fungal pathogens, this recognition was found to be inefficient, due to a lack of TLR costimulation, and contributed to chronicity of the infection (292). Moreover, in response to F. monophora, Mincle can suppress Dectin-1-mediated IL-12 production, promoting Th2 responses (178). Similarly, Mincle was shown to suppress Th17 cell differentiation induced by Dectin-2 (248).

Mincle also recognizes endogenous ligands. Spliceosome-associated protein (SAP) 130, released from necrotic cells, was shown to be a ligand for Mincle, although recognition occurred through a different binding site on its CTLD (262). Mincle recognition of SAP130 induces inflammatory cytokine production (MIP-2 and TNF, for example) and neutrophil accumulation (262). Recently, human Mincle was also shown to recognize cholesterol crystals (293). This recognition
of endogenous ligands suggests a role for Mincle in homeostasis, although our understanding of this function is still poor. There is emerging evidence, however, suggesting that Mincle may be involved in RA, pathogenesis of ischemic stroke and early brain injury after subarachnoid hemorrhage, and obesity-induced adipose tissue inflammation and fibrosis (294–298).

The Macrophage MR

The MR (CD206) is a type I transmembrane protein that contains a heavily glycosylated extracellular region consisting of a cysteine-rich domain, a fibronectin type II domain, and eight CTLDs (Fig. 4) (299, 300). Two conformations of the MR have been proposed: an extended form and a more compact “bent” form that is influenced by pH (301). The MR is expressed predominantly intracellularly, as part of the endocytic pathway, in subsets of macrophages and DCs, as well as some other nonmyeloid cell types including endothelial cells (300, 302). The expression of this receptor can be influenced by several cytokines, including IL-4, which causes marked upregulation of the MR (303). In fact, this upregulation has led to the MR being used as a marker for alternatively activated macrophages (304). Within the MR gene is a coregulated microRNA (miR-511-3p) that modulates cellular activation in tumor-associated and other macrophages and was recently shown to contribute to intestinal inflammation (305, 306). The extracellular domain of the MR can also be cleaved by metalloproteinases following cellular activation, through Dectin-1 signaling, for example, releasing a functional soluble form (sMR) (307).
The extracellular domains of the MR each recognize different structures. The cysteine-rich domain binds sulfated carbohydrates, the fibronectin domain binds collagen, while the CTLDs (specifically CTLDs 4 to 8) bind terminal mannose- and fucose-based structures as well as N-acetylglucosamine in a Ca\(^{2+}\)-dependent manner (300). The MR can also recognize CpG motif-containing oligodeoxynucleotides (308). The recognition of such a broad range of structures has led to substantial literature implicating the MR in both homeostasis and antimicrobial immunity. Indeed, the MR has been shown to recognize multiple types of pathogens, including viruses, helminths, trypanosomes, fungi, and bacteria (166, 300). Recognition by the MR has been proposed to induce several cellular responses, including endocytosis, phagocytosis, antigen cross-presentation, and cytokine production, and modulate the development of adaptive immunity (300, 309, 310). How the MR actually mediates many of these responses is unclear, as the receptor lacks known signaling motifs in its cytoplasmic tail, although its ability to mediate antigen cross-presentation was shown to involve ubiquitination (311). In fact, its role in some of these responses is now controversial. For example, the MR was initially described as a phagocytic receptor, but was subsequently shown not to be directly capable of mediating this activity (312, 313).

Several lines of evidence suggest that the effects ascribed to the MR may stem from collaboration with other receptors. For example, this receptor has been proposed to collaborate with Dectin-1 and the TLRs in the response to fungi such as C. albicans and Paracoccidioides brasiliensis, inducing the production of IL-17, Th17, and Tc17 cells (314–316). The differential responses to various MR ligands also support a notion for collaboration with other receptors from intracellular signaling. For example, mannann had no effect on DC cytokine production, yet other MR ligands, including mannose-capped lipoarabinomannan (a mycobacterial cell envelope molecule) and biglycan (an extracellular matrix proteoglycan), were found to influence cytokine responses in these cells (317). However, many of the ligands of the MR are recognized by other receptors, such as Dectin-2 and Mincle (discussed above), and this overlapping specificity casts doubt on much of the early work.

Despite the considerable literature implicating the MR in immunity, studies of MR-deficient mice have suggested that the functions of this receptor are largely redundant. These mice are viable and do not show significantly increased susceptibility to most infectious agents. For example, loss of the MR did not alter the susceptibility of mice to infection with M. tuberculosis, C. albicans, or Pneumocystis murina (318–320). On the other hand, the MR knockout mice were found to have slightly increased susceptibility to infections with C. neoformans, due to alterations in development of protective CD4 T-cell responses (321). Deficiency of the MR has also been shown to lead to alterations in the regulation of serum glycoprotein homeostasis and the development of crescentic glomerulonephritis, as well as allergic responses to cat allergens such as Fel D1 (322–324). In humans, polymorphisms in MR have been linked to susceptibility to asthma, sarcoidosis, and tuberculosis (325–327).

CONCLUSION

Research over the last few decades has provided exciting new insights into the wide and varied functions of lectins. Through their ability to recognize carbohydrates and other ligands, we now appreciate that these molecules are an essential component of multicellular existence. As our understanding of the physiological roles of these receptors increases, opportunities for novel therapeutic approaches are emerging, such as the targeting of these receptors to drive vaccine responses (328). Yet there is still much we need to learn. For example, we tend to study these molecules in isolation, but it is clear that these receptors function in a coordinated and cooperative fashion. Indeed, the recognition of intact pathogens involves numerous receptors that trigger multiple intracellular signaling pathways, producing an integrated cellular response. Despite the importance of such receptor cross talk, we still understand very little about how such signaling is integrated and how this directs the final immunological response. We also know relatively little about the regulation and influence of glycosylation on homeostasis and immune function or the recognition mechanisms that are involved. Tackling these important problems is a priority for future research.

ACKNOWLEDGMENTS

The authors thank the Wellcome Trust, Medical Research Council, and Arthritis Research UK for funding.

REFERENCES


Mucus enhances gut homeostasis and oral tolerance by delivering im-
Xiong H, Mayer L, Berin C, Augenlicht LH, Velcich A, Cerutti A.

167. Shan M, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K,
Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas

168. Thiagarajan PS, Yakubenko VP, Elsori DH, Yadav SP, Willard B,
Ariizumi K, Shen GL, Shikano S, Xu S, Ritter R, III, Kumamoto T,
authors. ASMscience.org/MicrobiolSpectrum 21

Winther MP, van den Berg TK. 2015. Dectin-1 deficiency does not affect

170. Rochereau N, Drocourt D, Perouzé E, Pavot V, Redelinguys P,
Mosciai A, Tschopp J, Ruland J.

171. Hess C, Reid DM, Majoul IV, Strait RT, Harris NL, Köhl G, Wex E, Ludwig


173. Seifert L, Deutsch M, Alothman S, Alqunaidib D, Werba G, Pansari


176. Rogers NC, Slack EC, Edwards AD, Nolte MA, Schulz O, Schweighoffer

177. Drummond RA, Brown GD. 2013. Signalling C-type lectins in anti-

178. Wevers BA, Kaptein TM, Zijistra-Willens EM, Theelen B, Boekhout T,
Cell Host 6:16043–16054.


181. Goodrich HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf

182. Dorhoi A, Desel C, Yeremeev V, Pradl L, Brinkmann V, Mollenkopf

183. del Fresno C, Soulat D, Roth S, Blazek K, Udalova I, Sancho D,
Ruland J, Ardavin C. 2013. Interferon-γ production via Dectin-1-

184. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large


186. Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Wevers B,
Briujs SC, Geijtenbeek TB. 2009. Dectin-1 directs T helper cell differ-


189. Goodrich HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf

190. Goodridge HS, Shimada T, Wolf AJ, Hsu YM, Becker CA, Lin X,


194. Mansour MK, Tam JM, Khan NS, Seward M, Davids PJ, Puranam S,

195. Gringhuis SI, Kaptein TM, Wevers BA, Theelen B, van der Vlist M,


Lectin Receptors Expressed on Myeloid Cells


Lectin Receptors Expressed on Myeloid Cells


