Current perspectives

The role of lectins in allergic sensitization and allergic disease

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Allergic diseases are a global public health issue affecting millions of persons around the world. However, full understanding of the molecular basis of this group of chronic inflammatory disorders remains rather elusive. Recently, the role of carbohydrates on allergens and their counterstructures on antigen-presenting cells (lectins) have been highlighted as crucial factors in allergen sensitization, which culminates in T_H2 cell differentiation and the production of deleterious specific IgE antibodies. Here we review recent progress on the role of different lectins in patients with type I hypersensitivity or allergy, their interplay with other determinants of allergenicity, and ways of developing therapeutic modalities against newly identified targets. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *C-type lectin receptor, lectin, glycosylation, allergen, type-I hypersensitivity, asthma, house dust mite, mannose receptor, DC-SIGN, Toll-like receptor, dendritic cells, galactins*

Abbreviations used

AHR:	Airway hyperresponsiveness
CCD:	Cross-reactive carbohydrate determinant
CD:	Cytoplasmic domain
CLR:	C-type lectin receptor
CRD:	Carbohydrate recognition domain
CTLD:	C-type lectin–like domain
DC:	Dendritic cell
DC-SIGN:	Dendritic cell–specific intracellular adhesion molecule 3–grabbing nonintegrin
Gal:	Galectin
HDM:	House dust mite
Man-LAM:	Mannose-capped lipoarabinomannan
MBL:	Mannose-binding lectin
MC:	Mast cell
MR:	Mannose receptor
OVA:	Ovalbumin
PRR:	Pattern recognition receptor
SP:	Surfactant protein
TLR:	Toll-like receptor

Type I hypersensitivity or allergy is an exacerbated immune response against specific antigens called allergens. The re-exposure to those molecules by means of inhalation, ingestion, injection, or direct contact triggers the allergic reaction characterized by the synthesis of IgE. In general terms the sequence of events starts with the recognition of an allergen by dendritic cells (DCs), followed by T_H2 cell differentiation, IgE production, and mast cell (MC) sensitization and triggering. During re-exposure, the cross-linking of Fc receptor–bound IgE on MCs by allergens promotes the release of soluble mediators and onset of the allergic reaction.[1] DCs have been shown to have a crucial role in the induction and re-elicitation of T_H2-mediated allergic diseases; however, the molecular processes underpinning these events are still unclear.[2]

Recognition and internalization of antigens by DCs is an important first step in the sequence of events that leads to the induction of the adaptive immune response. Immature DCs take up antigens in the periphery, process them into peptides, and then migrate to the lymph nodes, where, through expression of costimulatory molecules and cytokines, they can stimulate naive T cells or induce tolerance, depending on the nature of the antigen and other microenvironmental factors.[3] DCs efficiently sample their milieu for foreign antigens by using pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), or scavenger receptors, which increase their internalization efficiency and deliver information regarding the presence of danger signals.[4]

This review will focus on the role of different membrane-associated CLRs, soluble lectins, and galectins in allergen recognition and downstream events leading to T_H2 cell polarization. We will also discuss the interplay between lectins, PRRs, and other determinants of allergenicity, such as molecular mimicry and enzymatic activity, and how such interactions could collectively determine the outcome of the immune response to allergens.

MEMBRANE-ASSOCIATED CLRs

CLRs play a key role in antigen uptake by DCs and are particularly involved in the uptake of glycoantigens. A number of CLRs, including mannose receptor (MR), dendritic cell–specific intracellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN), and Dectin-2 have been recently shown to act as receptors for allergens.

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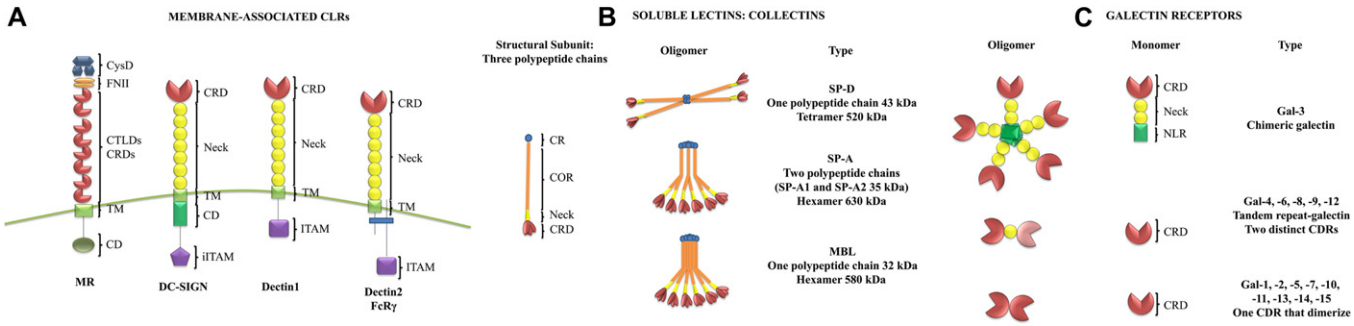

FIG 1. The domain structure of lectins: schematic representation of the polypeptide and domain structures of membrane-associated CLRs **(A)**,[5-8] soluble lectins **(B)**,[9] and galectins **(C)**.[10] *CRD*, Carbohydrate recognition domain; *COR*, collagen region; *CysD*, cysteine-rich domain; *FNII*, fibronectin type II–like domain; *iITAM*, incomplete immunoreceptor tyrosine-based activation motif; *NLR*, nonlectin region; *TM*, transmembrane region.

MR

Structure. The MR is a 175-kDa type I integral transmembrane glycoprotein with established roles in homeostasis and immunity. It recognizes a wide range of carbohydrates on microbial cell surfaces and mediates endocytic clearance of host-derived glycoproteins. The domain structure of the MR contains 3 regions: a cysteine-rich domain, a fibronectin type II–like domain, and 8 C-type lectin-like domains (CTLDs). These are followed by a transmembrane region and a short COOH terminal hydrophilic cytoplasmic domain (CD), which participates in receptor internalization and recycling (Fig 1, *A*).[5-10] The MR is a multifunctional receptor with 2 lectin activities involving Ca^{2+}-dependant recognition of carbohydrates terminated in L-fucose, D-mannose, or N-acetyl glucosamine through CTLDs, as well as Ca^{2+}-independent binding of acidic glycans sulfated at positions 3 or 4 through the cysteine-rich domain, whereas the fibronectin type II–like domain mediates collagen binding.[5]

Role in allergen recognition. Some data suggest an association between the MR and airway diseases. In a clinical study it was found that DCs from allergic patients expressed more MR and were also more efficient in the uptake of the house dust mite (HDM) allergen Der p 1.[11] Interestingly, gene-mapping linkage analyses in both human subjects[12] and mice[13] have identified *Mrc1* (MR C-type 1) as a positional candidate gene for allergen-induced airway hyperresponsiveness (AHR), which indicates a clear association between the MR and asthma. In line with these observations, recent data have shown that the MR on human DCs is a common receptor for several clinically relevant allergens, including those from HDMs (Der p 1 and Der p 2), cockroach (Bla g 2), dog (Can f 1), and peanut (Ara h 1), and that recognition of these allergens is mediated by the CTLD4-7 region of the MR (Table I).[14-23] Also, it was shown that the MR plays a crucial role in T_H2 cell polarization, as demonstrated by a biased T_H1 response when MR-deficient DCs were stimulated with Der p 1 and cocultured with naive T cells. Interestingly, the reversal of a biased T_H1/T_H2 balance in the absence of the MR was shown to be mediated, at least in part, through upregulation of indoleamine 2,3-dioxygenase activity in DCs,[14] an immune-modulatory enzyme that participates in tryptophan metabolism.[24] Later, it was shown that the MR was also an endocytic receptor for the uptake of the major cat allergen Fel d 1 and it mediated production of Fel d 1–specific IgE and IgG_1 in a mouse model of allergy.[15]

TABLE I. Interactions of lectins with various allergens

Lectin	Allergen	Source	Reference
MR	Der p 1	HDM	14
	Der p 2	HDM	14
	Bla g 2	Cockroach	14
	Can f 1	Dog	14
	Ara h 1	Peanut	14
	Fel d 1	Cat	15
DC-SIGN	Der p 1	HDM	18
	Der p 2	HDM	17
	Can f 1	Dog	18
	Ara h 1	Peanut	16
	BG-60	Pollen	17
Dectin-2	*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* extracts	HDM	19
	Aspergillus fumigatus extract	Mold	19
SP-A	Der p 1	HDM	22
	Der f 1	HDM	22
	Populus nigra var. italica, *Poa pratensis*, *Secale cereale*, and *Ambrosia artemisiifolia* var. elatior extracts	Pollen grains	20
	Glycoproteins 55 and 45 from *Aspergillus fumigatus*	Mold	23
SP-D	Der p 1	HDM	22
	Der f 1	HDM	22
	Dactylis glomerata and *Phleum pratense*	Pollen starch granules	21
	Glycoproteins 55 and 45 from *Aspergillus fumigatus*	Mold	23

DC-SIGN

Structure. DC-SIGN is a 44-kDa type II transmembrane protein receptor that is able to bind mannose- and fucose-containing ligands and is exclusively expressed by antigen-presenting cells.[25] Moreover, it functions as a cell adhesion receptor mediating migration and antigen internalization by DCs.[26] DC-SIGN consists of 3 regions: an extracellular domain

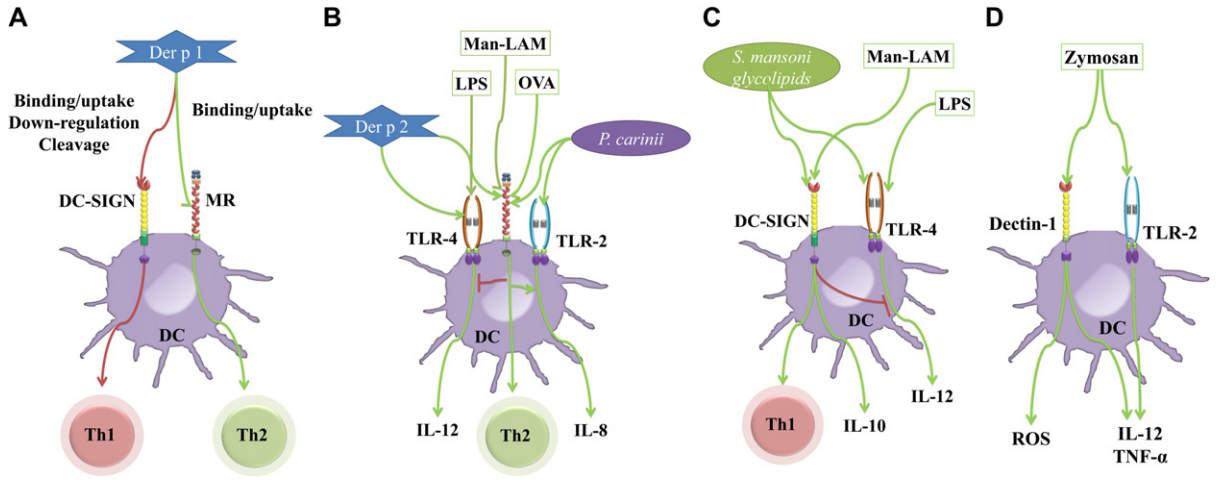

FIG 2. Interaction between CLRs and TLRs. **A,** Antagonistic effect between the MR and DC-SIGN. Both the MR and DC-SIGN have been shown to recognize and internalize Der p 1 but with opposite consequences.[14,18] Moreover, Der p 1 has been shown to downregulate the expression of DC-SIGN,[30] and it can also cleave DC-SIGN.[29] **B,** Interaction between the MR and TLRs. Binding of Man-LAMs to the MR inhibits the production of IL-12 after exposure to LPS.[36,38] Der p 2 binds to the MR on DCs, leading to a T_H2 response, as well as activating TLR4 signaling.[14,32] OVA mediates the upregulation of both the MR and TLR2 and is able to bind to the MR and possibly trigger these effects.[33] There is production of IL-8 after exposure to *P carinii* in a cell line that coexpresses the MR and TLR2.[31] **C,** Interaction between DC-SIGN and TLR4. The ligation of DC-SIGN with Man-LAMs inhibits the production of IL-12 after exposure to LPS but increases the production of IL-10.[34,39] Both DC-SIGN and TLR4 are involved in the response to *Schistosoma mansoni* glycolipids.[37] **D,** Interaction between Dectin-1 and TLR2. In macrophages the ligation of Dectin-1 alone with zymosan leads to the secretion of reactive oxygen species *(ROS)*, and simultaneous ligation of Dectin-1 and TLR2 enhances the secretion of IL-12 and TNF-α at levels higher than those induced by TLR2 alone.[35] Activating signals are shown in *green*, and inhibitory signals are shown in *red*.

that contains a carbohydrate recognition domain (CRD), a neck or hinge domain followed by a transmembrane region, and a CD (Fig 1, *A*).[6] The CRD forms part of 2 Ca^{2+}-binding sites, and it can recognize glycosylated antigens or carbohydrate structures, such as mannose-capped lipoarabinomannans (Man-LAMs) and Lewis-X, respectively.[27] The CD contains internalization motifs, such as dileucine triacidic clusters, and unlike the MR, it contains an incomplete immunoreceptor tyrosine–based activation motif.[28]

Role in allergen recognition. DC-SIGN has been shown to mediate the uptake of various allergens, such as the major peanut allergen (Ara h 1),[16] the Bermuda grass pollen allergen (BG-60), and the major group 2 allergen from HDM (Der p 2) by human DCs.[17] Recently, our group identified DC-SIGN on human DCs as a receptor for the major dog (Can f 1) and HDM allergens (Der p 1, Table I).[18] Moreover, intriguingly, it was shown that knockdown of DC-SIGN leads to a bias toward T_H2 polarization in autologous DC–T-cell cocultures. In contrast, our previous work showed that knocking down of the MR leads to an opposite effect (ie, bias in favor of T_H1[14,18]; Fig 2, *A*).[14,18,29-39] It is important to mention in this connection that Der p 1, through its cysteine protease activity, can cleave DC-SIGN but not the MR, and this could potentially further amplify its allergenicity.[29] Der p 1 is also known to induce downregulation of DC-SIGN expression during the differentiation of immature monocyte-derived DCs.[30] In keeping with these data, it is possible that the overall lineage fate of T cells in response to allergen exposure in different subjects could be determined at least partly by the relative levels of MR and DC-SIGN expression on DC subsets.[18] Therefore it is interesting to note that MR expression is reported to be higher in atopic subjects,[11,40] whereas DCs derived from Der p 1–sensitized asthmatic patients exhibits decreased expression of DC-SIGN.[30]

Dectin receptors

Dectins are type II transmembrane proteins receptors with an extracellular domain containing a highly conserved CRD (Fig 1, *A*). Dectin-1 and Dectin-2 are both expressed by DCs and have been implicated in infectious and allergic diseases. Dectin-1 is a receptor for yeast β-glucan,[7] whereas Dectin-2 is a PRR for fungi. Unlike Dectin-1, Dectin-2 does not have any signaling motif and uses an Fc receptor γ chain signal for internalization and activation of nuclear factor κB signaling, which leads to the upregulation of TNF-α and IL-1 receptor antagonist.[8] Furthermore, Dectin-2 has been shown to act as a receptor for HDM (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) and mold *(Aspergillus fumigatus)* extracts (Table I). Interestingly, the same study showed that recognition of allergen extracts by Dectin-2 could trigger cysteinyl leukotriene generation by murine DCs.[19] Later, it was shown that Dectin-2, through triggering the generation of cysteinyl leukotrienes, could mediate type 2 immune responses, including pulmonary inflammation, against HDM allergens.[41]

SOLUBLE LECTINS: COLLECTINS

Structure

The collectins are a family of mammalian lectins in which a CRD is attached to a collagen region through an α-coil domain (Fig 1, *B*). Although the CRD participates in the recognition of sugar moieties on the surface of glycoproteins on microorganisms or host cells, the neck region induces trimerization of the protein, which is further stabilized by the collagen domain. Higher oligomerization is promoted by the N-terminal domain.[9]

Role in allergen recognition: Surfactant proteins A and D

Lung surfactant proteins (SPs) A and D, which are synthesized by alveolar type II cells, are among the 6 human collectins that have been described. Apart from their role in surfactant homeostasis, they have been demonstrated to take part in protection against allergens and respiratory pathogens.[42] Both SP-A and SP-D have been shown to mediate the binding of pollen grains and pollen-allergen starch granules to alveolar type II cells and macrophages, enhancing their phagocytosis.[20,21] They can also bind directly to HDM extract, purified Der p 1, and glycoprotein allergens from *A fumigatus* in a carbohydrate-specific and Ca^{2+}-dependent manner, inhibiting specific IgE binding to these glycoprotein allergens, blocking allergen-induced histamine release from basophils, and reducing the proliferation of PBMCs isolated from Der p 1–sensitive asthmatic children (Table I).[22,23,43]

Interestingly, 2 major mite allergens, Der p 1 and Der f 1, were shown to interact with SP-A and SP-D, whereby these allergens, using their cysteine protease activity, could cleave and inactivate both SPs. This was associated with diminished binding to carbohydrates and reduced capacity to agglutinate bacteria, compromising a potential innate immune defense mechanism against allergens.[44]

In vivo studies and putative mechanisms

One study found that SP-$D^{-/-}$ mice were more vulnerable than wild-type mice to *A fumigatus* sensitization, whereas SP-$A^{-/-}$ mice were virtually resistant.[45] A previous study with a murine model of pulmonary inflammation showed that SP-D only participates in an initial resistance to ovalbumin (OVA) allergen.[46] Accordingly, it could be speculated that SP-A and SP-D can modulate allergic responses through different mechanisms. Related to that, it has been shown that IL-13 is a potent stimulator of SP-D in the lung, increasing up to 70-fold in a murine model of IL-13 overexpression.[47] However, other data suggest that during lung inflammation, SP-A and SP-D exert an immune-balancing function.[48,49] SP-A has been shown to inhibit LPS-mediated surface expression of maturation markers on immature DCs and allostimulation on T cells,[48] and SP-D mediates the binding and uptake of *Escherichia coli* by bone marrow–derived mouse DCs, which lead to increased antigen presentation.[49] The ability of SP-A and SP-D to decrease specific IgG and IgE levels could be explained by their potential to reduce the proliferation of specific B cells.[50] This effect on B cells can be further intensified by a reduction in IL-2 levels because IL-2 plays a crucial role in lymphocytes growth and differentiation[51]; this has been reviewed by Kishore et al.[9,52] However, shifting cellular responses to a T_H1 profile seems to be central in SP-A and SP-D protective mechanisms, whereby IFN-γ promotes cellular immunity and blocks IL-4–mediated T_H2 differentiation, which, together with IL-13, is very important for isotype switching of B lymphocytes.[9]

Clinical relevance of mannose-binding lectin

Finally, a link has been established between the plasma levels of the collectin mannose-binding lectin (MBL) and susceptibility to allergic responses.[53] Specifically, it was shown that plasma MBL levels and complement activity correlated with peripheral blood eosinophilia in patients with asthma, bronchial and allergic rhinitis, and allergic bronchopulmonary aspergillosis, which was further corroborated in a mouse model of *A fumigatus* hypersensitivity.[53] Moreover, one study identified a new polymorphism in an intronic zone of the MBL gene, which was associated with an increase in plasma MBL levels and eosinophil counts in patients with bronchial asthma.[54] On this basis, the authors suggested that the high levels of MBL might contribute to the increase in complement activation and eosinophilia.[55]

GALECTIN RECEPTORS

Structure

Galectins are a family of proteins that bind β-galactosides. These proteins are highly conserved and are expressed by diverse cell types, including monocytes, DCs, macrophages, MCs, and B and T cells. There are 3 types of galectins: galectins that consist entirely of 1 CRD, such as galectin (Gal) 1, Gal-2, Gal-5, Gal-7, Gal-10, Gal-11, Gal-13, Gal-14, and Gal-15; tandem repeat galectins, such as Gal-4, Gal-6, Gal-8, Gal-9, and Gal-12, which contain 2 homologous CRDs separated by a linker; and chimeric galectins, such as Gal-3, which contain a CRD preceded by a nonlectin region consisting of short Pro/Gly-rich tandem repeats (Fig 1, *C*).[10] Galectins can function either extracellularly or intracellularly. In terms of extracellular functions, galectins can be involved in cell activation, cell adhesion, migration, cell growth, phagocytosis and apoptosis. On the other hand, intracellularly, they can participate in the signaling pathway, gene expression, and vesicular trafficking, among others.[56,57]

Role of Gal-3

A number of galectins, particularly Gal-3 and Gal-9, have been shown to play an important role in T_H2-mediated immune responses. For example, in a mouse model of asthma, it was found that OVA-sensitized Gal-$3^{-/-}$ mice had less goblet cell metaplasia, lower eosinophilia, less AHR, and a lower T_H2 response compared with Gal-$3^{+/+}$ mice.[58] The contribution of Gal-3 in T_H2 responses was further corroborated in murine models of atopic dermatitis and chronic allergic inflammation.[59] Moreover, it was shown that bone marrow–derived MCs,[60] DCs,[61] and leukocytes[62] from Gal-$3^{-/-}$ mice exhibited a T_H1-polarized response compared with cells from Gal-$3^{+/+}$ mice. Interestingly, a study in patients with cow's milk allergy has shown that their duodenal intraepithelial lymphocytes preferentially bind Gal-3 expressed by intestinal epithelial cells and macrophages compared with healthy control subjects.[63] These data clearly suggest a potential role for Gal-3–glycan interactions in modulating the epithelial–immune cell cross-talk during allergic inflammation.[63]

Role of Gal-9

Gal-9 can act as an autocrine regulator of the effector functions of MCs. For instance, it is able to bind IgE efficiently and block the formation of the IgE-allergen complex, leading to blockage of MC degranulation and alleviation of asthmatic reactions in an experimental model of asthma.[64] Furthermore, in a mouse model of mite allergen–induced asthma, the therapeutic effect of Gal-9 was demonstrated by showing reduced AHR, as well as T_H2-associated airway inflammation.[65] By contrast, in a guinea pig asthma model, it was shown that Gal-9 is not involved in AHR but is partially involved in prolonged eosinophil accumulation

in the lung.[66] The same was demonstrated in an OVA-induced mouse model of allergic asthma, whereby it was postulated that Gal-9 might serve as a recruiter of eosinophil granulocytes, hence promoting dominant T_H2 responses.[67]

ROLE OF SUGAR MOIETIES ON ALLERGENS

There are 2 main types of carbohydrates, N-linked and O-linked. The initial steps of protein N-glycosylation are essentially conserved in all eukaryotic organisms, but variations between vertebrates and invertebrates are often great, resulting in immunogenic structures. However, O-linked glycans, despite having a large number of modifications, are very similar in invertebrates and vertebrates.[68]

Role of N-glycosylation

The asparagine-linked sugar moieties of plant and insect glycoproteins, which are the most abundant environmental immune determinants, form the structural basis of what is called cross-reactive carbohydrate determinants (CCDs). In spite of some variation, the 2 main epitopes are the core-3-linked fucose and xylose.[69] Recently, we showed that both Der p 1 and Der p 2 contain 1-3 fucose linked to asparagine.[70]

There is a full body of data indicating that IgE anti-CCDs are involved in *in vitro* reactivity of patients' sera to a wide variety of allergens, mainly from insect venoms, grass and tree pollens, and foods.[69] In addition, *in vitro* reports have shown that glycoproteins and IgE anti-CCD induce the release of histamine[71,72] and IL-4 from basophils,[73] even though CCDs do not seem to cause clinical symptoms in most patients. This benign nature of CCDs can be explained by the interception of IgE binding by blocking antibodies, presumably IgG_4, which is also an important mechanism contributing to the efficacy of antiallergy vaccination or specific immunotherapy.[74] Moreover, there is evidence that those antibodies can be induced by an incidental immune therapy exerted by everyday contact with plant materials.[75]

The role of sugar moieties in allergen recognition has been addressed in different ways. Some studies have used either chemical (periodate sodium) or enzymatic (glycosidases) treatments for destroying carbohydrate determinants on allergens.[76] Recent studies have shown that the uptake of periodate sodium-treated (ie, deglycosylated) Der p 1 by DCs was minimal compared with the uptake of hyperglycosylated recombinant and natural counterparts.[70] Periodate treatment did not seem to affect the structural integrity of Der p 1.[70] However, in spite of some encouraging results,[70,77] chemical deglycosylation is not specific and can potentially distort the protein structure,[78] whereas glycosidases are more specific but less effective than chemical methods.[79] That is why the use of recombinant nonglycosylated allergens could be the best way to approach those studies.[69] Accordingly, some studies have shown significant differences, such as low binding to IgE[72,78] and less induction of histamine release,[71,72] by the recombinant nonglycosylated form of the allergen compared with its native counterpart, which demonstrates an important role for sugar moieties on allergens. In addition, recombinant structures have been used to determine the specificity of IgG and IgE antibodies,[80] to test their biological activity by means of intracutaneous skin testing,[81] and to induce T cell–mediated and humoral *in vitro* and *in vivo* responses.[82] In this context recombinant allergens and hypoallergenic derivatives thereof have also been used in diagnostics and as vaccines in clinical trials, and some studies have shown their effectiveness for the treatment of type I hypersensitivity.[69,83]

Role of O-glycosylation

In the case of O-glycans, some new determinants have been identified in the major mugwort allergen (Art v 1).[78] However, the incidence of such O-glycan epitopes appears to be restricted,[69] in some cases without any biological significance.[73]

OTHER DETERMINANTS OF ALLERGENICITY AND THEIR CROSS-TALK WITH CLRs

Protease activity

In addition to glycosylation, there are other properties, such as protease activity[1] and TLR mimicry,[32] that could render some proteins allergenic. Proteases from different allergens, such as cockroach, pollen, and HDM, have been shown to disrupt the epithelial tight junction and in doing so increase the permeability of the airway epithelium to allergens and other bystander antigens.[84-86] In the case of Der p 1, there are extensive data showing that its proteolytic activity could bias immune responses toward a T_H2 phenotype.[1] In addition to disturbing innate immune defenses at epithelial surfaces (eg, degradation of SP-A and SP-D[44] and cleavage of tight junctions[86]), Der p 1, in its enzymatically active form, has been shown to cleave different surface molecules, such as CD23 on B cells, CD25 on T cells, and CD40 and DC-SIGN on DCs, all of which are thought to contribute to and propagate Der p 1's allergenicity.[1] In the context of DC-SIGN, we had previously hypothesized that given the preferential role of intercellular adhesion molecule 3, the main DC-SIGN counterstructure, in T_H1 differentiation, DC-SIGN cleavage by Der p 1 could bias T-cell differentiation toward a T_H2 phenotype by compromising signaling through intercellular adhesion molecule 3.[29] More recently, we have shown that silencing DC-SIGN expression on human DCs could bias T-cell differentiation toward a T_H2 phenotype in DC–T-cell coculture experiments.[18] Therefore it is intriguing that Der p 1 is able to cleave its T_H1-promoting lectin receptor (DC-SIGN) but cannot do so for its T_H2-promoting receptor (MR), possibly because of the presence of a short neck region in the MR (Fig 2, *A*).[29] It is also reasonable to hypothesize that Der p 1 binding to DC-SIGN could be a prerequisite for DC-SIGN cleavage, which could in turn propagate T_H2 polarization.

TLR mimicry

Der p 2, another major allergen from HDM, has been shown to have structural and functional homology with MD-2, which is the LPS-binding component of the TLR4 signaling complex. This gives Der p 2 an intrinsic adjuvant property and the ability to directly interact with TLR4 complex and facilitate LPS signaling through TLR4, which is thought to underpin Der p 2's allergenicity.[32] It is interesting to note that Der p 2 has also been shown to be glycosylated, and its uptake by DCs is mediated through MR, at least in part (Fig 2, *B*).[14] Therefore a synergy between binding of Der p 2 to the MR and its functional mimicry to MD-2 is conceivable. Within this context, it is worth highlighting that TLR4 signaling and LPS exposure, most likely in a MyD88-dependent

manner, have been shown to play a key role in T_H2-mediated inflammation and asthma.[87-91]

On the other hand, CLRs act as "noncanonical" PRRs because they can facilitate access to and/or modulate PRR-induced responses. Accordingly, different CLRs, such as the MR,[33] DC-SIGN,[34] Dectin-1,[35] and Gal-9,[92] have been linked to TLR, particularly TLR4, signaling in T_H2 allergic responses (Fig 2). For example, unlike other CLRS, the MR does not have any signaling motif in its CD (Fig 1, *A*)[93]; however, it has been shown to participate in nuclear factor κB–mediated gene expression,[94] most likely through cross-talk with other receptors. Indeed, it has been demonstrated that both the MR and DC-SIGN could interact and synergize with TLR2[31,33] and TLR4[14,32] and in doing so facilitate signal transduction and subsequent events that lead to T_H2 and T_H1 polarization, respectively (Fig 2, *B* and *C*).[14,31-33,36,37,95] For example, both the MR[36,38] and DC-SIGN[34,39] have been shown to inhibit IL-12 production after ligation with Man-LAM, a member of *Mycobacterium tuberculosis* modulins, and subsequent challenge with LPS. In 2 independent experiments it was demonstrated that the MR and TLR2 can also converge in the recognition of *Pneumocytis carinii*[31] and the effects mediated by OVA involving the notch 1 signaling pathway in mouse DCs (Fig 2, *B*).[33] Both DC-SIGN and TLR4 have been shown to be involved in the response to *Schistosoma mansoni* glycolipids (Fig 2, *C*).[37] Finally, the ligation of zymosan by Dectin-1 and TLR2 alone or simultaneously on macrophages leads to different effects (Fig 2, *D*).[35] Collectively, these data provide evidence for synergistic interactions between different determinants of allergenicity, which most likely work in concert to bias immune responses toward a T_H2 phenotype after allergen exposure.

IMMUNOTHERAPY BASED ON LECTINS

Given their obvious role in the initiation and propagation of allergic responses, lectins could be attractive targets for the treatment and modulation of T_H2-type responses. This notion is already supported by a number of studies. For example, intratracheal gene therapy with Gal-3 has been shown to inhibit inflammation and bronchial obstruction in antigen-challenged rats through downregulation of the IL-5 gene,[96] even in a chronic model of inflammation.[97] On the other hand, other studies have shown that intranasal delivery of SP-A and SP-D decreases allergen-specific antibody levels and eosinophil counts, as well as skewing immune responses toward a T_H1 profile in a mouse model of allergic bronchopulmonary aspergillosis.[98,99] Similar protective effects of SP-D in murine models of HDM-induced pulmonary allergy were observed.[100,101] Finally, in an OVA-induced murine model of pulmonary inflammation and AHR, prophylactic intratracheal delivery of rat SP-D was shown to reduce AHR, eosinophilia, and goblet cell hyperplasia.[102]

In a recent development particles that can protect allergens from digestion and support intestinal antigen uptake were produced and used for oral immunotherapy of type I allergy. Birch pollen allergens were entrapped in microspheres, which were further coated with wheat germ agglutinin to target the sialylic residues on murine enterocytes. Feeding of BALB/c mice with coated microspheres induced higher levels of allergen-specific IgG than gavages of uncoated microparticles or naked protein.[103] In a subsequent study the same group demonstrated that when BALB/c mice are first sensitized to birch pollen and subsequently fed with birch pollen–loaded functionalized *Aleuria aurantia* microspheres to target α-L-fucose on M cells, birch pollen–specific IgG_{2a}, but not IgG_1 or IgE, levels increased significantly. Also, IFN-γ synthesis was significantly increased, which might have been responsible for the significant IgG_{2a} production.[104]

CONCLUSIONS AND FUTURE PERSPECTIVES

As demonstrated here, lectins and carbohydrates play a key role in allergic responses (Fig 3).* Between them, they are able to exert synergistic or antagonistic effects. For instance, the MR and DC-SIGN both are able to recognize and internalize Der p 1; however, those interactions lead to T_H2[14] and T_H1[18] responses, respectively. In addition, Der p 1 can cleave DC-SIGN and downregulate its expression on DCs,[29,30] whereas it does not have such effect on MR.[29] Dectin-2 can recognize different extracts from HDM and induce the release of cysteinyl leukotriene.[19] Furthermore, it is crucial in eosinophilic and neutrophilic pulmonary inflammation and T_H2 cytokine production.[41] Both SP-A and SP-D have been shown to bind HDM extracts and in that way block PBMC proliferation[9]; however, Der p 1 can degrade and inactivate both SP-A and SP-D, favoring an allergic response.[44] Furthermore, SPs can reduce the proliferation of B cells and shift the response to a T_H1 profile.[9] Finally, galectins are involved in epithelial cell–lymphocyte interactions[63] and the blocking of asthmatic reaction driven by IgE.[64]

The general physiologic functions of sugar moieties and their receptors are highly diverse and include roles in cell trafficking[105] and cellular signaling,[106] among others. Moreover, the lectin repertoire, as well as the cellular glycosylation signatures, participate in diverse cellular mechanisms involving innate and adaptive immune responses,[107] such as pathogen recognition,[108] antigen presentation,[109] immune tolerance,[110] and cancer progression[111] (Fig 4). In the case of galectins, they have been shown to be involved in different biological processes. In particular, they can regulate various mediators of cellular signaling through the cross-linking of glycoproteins,[106] mediate rolling and adhesion of eosinophils in cell trafficking,[105] modulate cancer progression,[111] and induce immune tolerance.[110] In addition, DC-SIGN has been demonstrated to be involved in antigen presentation.[109]

Unlike nucleic acids and proteins, carbohydrates remain an enigmatic arm of biology. Although carbohydrates play as diverse a function in biology as proteins, they have been difficult to study because of the complexity of their synthetic pathways, unlike the template-driven synthesis of nucleic acids and proteins. Our new insights into the role of lectins in the initial recognition and uptake of allergens by DCs could also be exploited in designing new intervention strategies aimed at early events (ie, allergen uptake by DCs) at the interface of allergens and innate immune cells. For example, localized blocking of allergen receptors, such as the MR, is likely to impede allergic sensitization and the development of symptoms. Moreover, mannose seems to be the dominant type of sugar carried on a diverse range of allergens, such as bromelain, papain, Bla g 1, Ara h 1, Can f 1, Fel d 1, and Der p 1.[70] Thus the development of different allergen glycoforms with immunemodulatory properties could be an alternative strategy for allergen-specific immunotherapy.[112]

In this review we propose that sugar moieties on allergens play an important role in allergen recognition. We suggest that their

*See references 9, 14, 18, 19, 29, 30, 41, 44, 63, and 64.

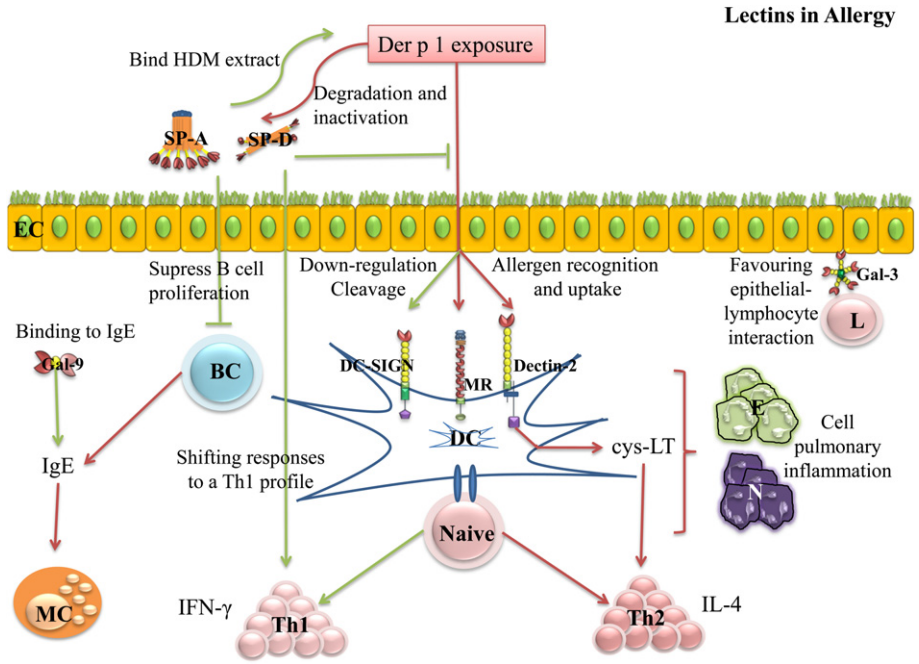

FIG 3. The role of lectins in allergy: synergistic and antagonistic effects of lectins in allergy. The MR is able to recognize and internalize Der p 1, leading to a T_H2 response.[14] DC-SIGN can also bind and internalize Der p 1, but that engagement leads to a T_H1 response.[18] Moreover, Der p 1 can cleave DC-SIGN and downregulates its expression on DCs as well.[29,30] Dectin-2 can recognize different extracts from HDMs and triggers cysteinyl leukotriene *(cys-LT)*.[19] Furthermore, Dectin-2 is crucial in eosinophilic *(E)* and neutrophilic *(N)* pulmonary inflammation and T_H2 cytokine production.[41] Both SP-A and SP-D have been shown to bind HDM extracts and in that way block the proliferation of PBMCs.[9] Moreover, they reduce the proliferation of B cells *(BC)* and shift the response to a T_H1 profile.[9] On the other hand, Der p 1 can degrade and inactivate both SP-A and SP-D, favoring an allergic response.[44] Finally, Gal-3 has been shown to enhance epithelial cell–lymphocyte *(EC-L)* interactions, possibly through its glycans.[63] On the other hand, Gal-9 is able to bind IgE and in that way blocks the asthmatic reaction.[64] *Red arrows* represent proallergic responses, and *green arrows* represent antiallergic responses.

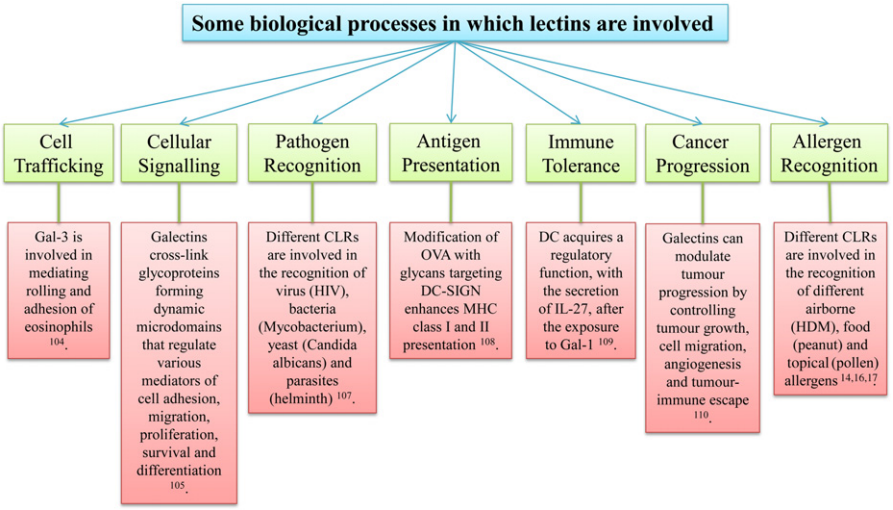

FIG 4. Some immune and nonimmune processes in which lectins are involved. Lectins participate in different immune and nonimmune processes, such as cell trafficking, cellular signaling, pathogen recognition, antigen presentation, immune tolerance, cancer progression, and allergen recognition.

presence on allergens is crucial in the allergen sensitization process because they participate in the recognition, uptake, and presentation of different glycosylated allergens on antigen-presenting cells. We also suggest that lectins recognize glycoallergens from diverse sources and that this engagement elicits different intracellular and extracellular responses, which in some cases lead to opposing effects (eg, the MR vs DC-SIGN). Some of these interactions could form the basis for developing

new strategies for immunotherapy of allergy. For instance, blocking allergen recognition and uptake by lectins, such as the MR, could be one strategy. On the other hand, DC-SIGN could be exploited in promoting antiallergic responses to switch the response to a protective T_H1 profile.

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