

MINIREVIEW

Lectins and cytokines in celomatic invertebrates: two tales with the same end**D Malagoli¹, S Sacchi², E Ottaviani¹**¹*Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy*²*Department of Biological Sciences, George Washington University, Washington DC, USA**Accepted December 18, 2009***Abstract**

The paper presents the principle data regarding the presence and the roles of lectins and cytokines in invertebrates. The former have been described in the main invertebrate taxa, such molluscs, annelids, arthropods, echinoderms and tunicates, while convincing evidence for cytokines was found only in the insects, *Drosophila melanogaster* and *Pseudaletia separata*, and the freshwater crayfish, *Pacifastacus leniusculus*. Lectins and cytokines share convergent and common functions, and one of the multiples roles of these messenger molecules is their participation in fighting against non-self.

Kew Words: invertebrate immunity; lectins; cytokines; evolution**Introduction**

According to Barondes (1988) e Yoshizaki (1990), lectins are sugar-binding proteins or glycoproteins bearing one or more sugar-binding sites and capable of agglutinating cells and/or precipitating glycoconjugates. The specificity of lectin is usually defined in terms of the mono-saccharide(s) or simple oligosaccharides that inhibit lectin-induced agglutination. Although lectins were first discovered in plants, they are present in all kingdoms, including bacteria and animals.

Cytokines constitute a more heterogeneous group of soluble mediators, but despite significant differences in terms of structure and function, some common characteristics are evident. Cytokines are mainly produced by the cells of the immune or neuroendocrine systems (Nisticò, 1993; Schöbitz *et al.*, 1994). Described principally in mammalian models, cytokines are glycoproteins of a relatively small molecular weight, and in most cases they are synthesized *de novo* by activated cells during the efferent phase of immune response. The main role of cytokines is that of mediator and modulator of immune responses and inflammation, but they are also involved as signal molecules in the neuroendocrine system (Nisticò, 1993; Blalock, 1994; Schöbitz *et al.*, 1994). Cytokines are characterized by pleiotropicity and redundancy (i.e., the same cytokine can have different effects on

diverse cellular targets, and the same function can be performed by different cytokines), they act on target cells by autocrine, paracrine and endocrine mechanisms, and they bind to specific plasma membrane receptors which show a certain degree of promiscuity (Kishimoto *et al.*, 1994; Paul and Seder, 1994). Notably, several human cytokines also display a lectin-like activity, and this may be essential to explain some of their biological properties (Cebo *et al.*, 2002).

Immune recognition in celomatic invertebrates is principally carried out by cells and humoral components that include lectins and cytokines and are present in the hemolymph (Ottaviani, 2005, 2006).

From the literature, it emerges that comparative immunologists have devoted their attention mainly to invertebrate lectins (Table 1) rather than to cytokines (Table 2). This may be related to the fact that, usually, lectins are more abundant, stable and functionally recognizable than cytokines and it is possible to purify and characterize a lectin even in absence of a conspicuous molecular dataset. Conversely, the isolation and characterization of a cytokine needs several molecular biology-based investigations. Now that molecular databases are becoming available for several invertebrate models, cytokines are, not surprisingly, receiving the appropriate attention.

Lectins

Lectins may be classified by structural or functional criteria. Lectins play an important role in cell-to-cell or cell-to-matrix interaction, glycoprotein

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Table 1 Examples of lectins described in invertebrates

Taxon	Species	Reference
MOLLUSCA	<i>Helix pomatia</i>	Hammarström and Kabat, 1969
	<i>Helix pomatia</i>	Hu <i>et al.</i> , 2008
	<i>Aplysia californica</i>	Pauley <i>et al.</i> , 1971
	<i>Mercenaria mercenaria</i>	Arimoto and Tripp, 1977
	<i>Biomphalaria glabrata</i>	Stein and Basch, 1979
	<i>Biomphalaria glabrata</i>	Boswell and Bayne, 1984
	<i>Mytilus edulis</i>	Renwrautz <i>et al.</i> , 1985
	<i>Octopus vulgaris</i>	Rögener <i>et al.</i> , 1985
	<i>Planorbarius corneus</i>	Ottaviani and Tarugi, 1986
	<i>Planorbarius corneus</i>	Ottaviani and Tarugi, 1986
<i>Crassostrea virginica</i>	Yamaura <i>et al.</i> , 2008	
ANNELIDA	<i>Lumbricus terrestris</i>	Stein <i>et al.</i> , 1982
	<i>Eisenia fetida</i>	Eue <i>et al.</i> , 1998
	<i>Caenorhabditis elegans</i>	Cooper and Barondes, 1999
	<i>Eisenia fetida</i>	Bloc <i>et al.</i> , 2002
ARTHROPODA	<i>Sarcophaga peregrina</i>	Komano <i>et al.</i> , 1980
	<i>Sarcophaga peregrina</i>	Takahashi <i>et al.</i> , 1985
	<i>Rhodnius prolixus</i>	Pereira <i>et al.</i> , 1981
	<i>Limulus polyphemus</i>	Rostam-Abadi and Pistole, 1982
	<i>Limulus polyphemus</i>	Muta <i>et al.</i> , 1991
	<i>Limulus polyphemus</i>	Amstrong <i>et al.</i> , 1996
	<i>Aphonopelma chalcodes</i>	Vasta and Cohen, 1984
	<i>Aphonopelma cochise</i>	Vasta and Cohen, 1984
	<i>Aphonopelma chiricawa</i>	Vasta and Cohen, 1984
	<i>Cancer antennarius</i>	Ravindranaths <i>et al.</i> , 1985
	<i>Spodoptera exigua</i>	Pendland and Boucias, 1986
	<i>Megabalanus rosa</i>	Muramoto and Kamiya, 1990
	<i>Periplaneta americana</i>	Jomori and Natori, 1991
	<i>Calliphora vomitoria</i>	McKenzie and Preston, 1992
	<i>Pacifastacus leniusculus</i>	Kopáček <i>et al.</i> , 1993
	<i>Tachypleus tredenatus</i>	Saito <i>et al.</i> , 1997
	<i>Tachypleus tredenatus</i>	Kawabata and Iwanaga, 1999
<i>Pinellia ternata</i>	Yao <i>et al.</i> , 2003	
<i>Anopheles gambiae</i>	Pace and Baum, 2004	
<i>Drosophila melanogaster</i>	Pace and Baum, 2004	
ECHINODERMATA	<i>Anthocidaris crassispina</i>	Giga <i>et al.</i> , 1985
	<i>Anthocidaris crassispina</i>	Giga <i>et al.</i> , 1987
	<i>Anthocidaris crassispina</i>	Ozeki <i>et al.</i> , 1991
	<i>Holothuria polii</i>	Canicatti and Rizzi, 1991
	<i>Asterina pectinifera</i>	Kamiya <i>et al.</i> , 1992
	<i>Paracentrotus lividus</i>	Canicatti <i>et al.</i> , 1992
	<i>Paracentrotus lividus</i>	Drago <i>et al.</i> , 2009
	<i>Stichopus japonicus</i>	Hatakeyama <i>et al.</i> , 1993
<i>Stichopus japonicus</i>	Himeshima <i>et al.</i> , 1994	

Table 1 (continue) Examples of lectins described in invertebrates

Taxon	Species	Reference
ECHINODERMATA	<i>Stichopus japonicus</i>	Matsui <i>et al.</i> , 1994
	<i>Cucumaria echinata</i>	Hatakeyama <i>et al.</i> , 1994
	<i>Cucumaria japonica</i>	Bulgakov <i>et al.</i> , 2000
	<i>Strongylocentrotus purpuratus</i>	Hibino <i>et al.</i> , 2006
	<i>Holothuria scabra</i>	Gowda <i>et al.</i> , 2008
TUNICATA	<i>Botrylloides leachii</i>	Coombe <i>et al.</i> , 1982
	<i>Didemnum candidum</i>	Vasta <i>et al.</i> , 1986
	<i>Phallusia mamillata</i>	Parrinello and Arizza, 1989
	<i>Styela clava</i>	Kelly <i>et al.</i> , 1992
	<i>Clavelina picta</i>	Elola and Vasta, 1994
	<i>Clavelina picta</i>	Vasta <i>et al.</i> , 1999
	<i>Botryllus schlosseri</i>	Ballarin <i>et al.</i> , 1999
	<i>Botryllus schlosseri</i>	Gasparini <i>et al.</i> , 2008
	<i>Halocynthia roretzi</i>	Sekine <i>et al.</i> , 2001
	<i>Pyura stolonifera</i>	Pearce <i>et al.</i> , 2001
	<i>Ciona intestinalis</i>	Azumi <i>et al.</i> , 2003
	<i>Ciona intestinalis</i>	Parrinello <i>et al.</i> , 2007
	<i>Ciona intestinalis</i>	Bonura <i>et al.</i> , 2009

trafficking, protein folding, signal transduction, fertilization, development and self/non-self discrimination (Vasta *et al.*, 2004). With regards the structural composition, at least seven families have been identified in animals on the basis of the carbohydrate-recognition domain (CRD): 1. P-type lectins; 2. S-type lectins; 3. C-type lectins; 4. pentraxins (Sharon, 1993; Drickamer and Taylor, 1993); 5. I-type lectins (Gabius, 1997; Angata *et al.*, 2002); 6. fucoselectins (Bianchet *et al.*, 2002); 7. rhamnose-binding lectins (Jimbo *et al.*, 2007; Terada *et al.*, 2007). Further studies have revealed others, e.g., galectins (formerly included among S-type lectins) (Barondes *et al.*, 1994), calnexin, calreticulin (Trombetta and Helenius, 1998; Parodi, 2000), collectins, ficolins (Lu *et al.*, 2002), immunlectins (ascribable to C-type lectins) (Yu *et al.*, 2002) and mannose-binding lectins (ascribable to C-type lectins) (Ip *et al.*, 2009).

Vasta and colleagues (2004) report that only some of the above mentioned lectins are present in invertebrates. The C-type CRDs have been reported in several invertebrates, such as the flesh fly *Sarcophaga peregrina* (Takahashi *et al.*, 1985), the sea urchin *Anthocidaris crassispina* (Giga *et al.*, 1987), the acorn barnacle *Megabalanus rosa* (Muramoto and Kamiya, 1990), the tunicate *Polyandrocarpa misakiensis* (Suzuki *et al.*, 1990), the horseshoe crab *Limulus polyphemus* (Muta *et al.*, 1991), the cockroach *Periplaneta americana* (Jomori and Natori, 1991), the sea urchins *Paracentrotus lividus* (Canicatti *et al.*, 1992) and *Strongylocentrotus purpuratus* (Smith *et al.*, 1996) and the sea cucumber *Stichopus japonicus* (Himeshima *et al.*, 1994). Pentraxins have been

reported in the tunicates *Clavelina picta* (Elola and Vasta, 1994), the horseshoe crabs *L. polyphemus* (Amstrong *et al.*, 1996) and *Tachypleus tridenatus* (Saito *et al.*, 1997). Galectins were found in the dipterans *Drosophila melanogaster* and *Anopheles gambiae* (Pace and Baum, 2004), the nematode *Caenorhabditis elegans* (Cooper and Barondes, 1999) and the ascidian, *Clavelina picta* (Vasta *et al.*, 1999). Fucoselectins have been retrieved in bivalves (Yamaura *et al.*, 2008) and rhamnose-binding lectins have been observed in bivalves (Naganuma *et al.*, 2006), echinoderms (Ozeki *et al.*, 1991) and tunicates (Gasparini *et al.*, 2008).

If the increased availability of molecular information has improved our knowledge of invertebrate cytokines, this is also the case for lectins. For instance, two galectins have been characterized in *C. elegans* and a screening of the GenBank database ten years ago retrieved 26 putative galectins (Cooper and Barondes, 1999). In the fruit fly *D. melanogaster* a galectin homologue (Dmgal, GenBank accession number AF338142) has been identified (Pace *et al.*, 2002), and in the solitary ascidian *Ciona intestinalis* nine collectin-like genes have been retrieved (Azumi *et al.*, 2003). Collectin gene expression has been found to change after LPS injection in *C. intestinalis* (Bonura *et al.*, 2009). In the colonial ascidian *Botryllus schlosseri* Ballarin and collaborators have identified 5 transcripts from a cDNA library, each with a complete coding sequence homologous to known rhamnose-binding lectins (Gasparini *et al.*, 2008). In the fully sequenced genome of *S. purpuratus* were identified 104 genes that encode for small C-type lectins composed of one or two domains that can

Table 2 List of the cytokines described in invertebrates, including DHF

Taxon	Species	Cytokine name	Reference
ARTHROPODA	<i>Drosophila melanogaster</i>	Spätzle	Morisato and Anderson, 1994
	<i>Drosophila melanogaster</i>	Upd-3	Agaisse <i>et al.</i> , 2003
	<i>Drosophila melanogaster</i>	DHF	Malagoli <i>et al.</i> , 2007
	<i>Pseudaletia separata</i>	HCP	Nakatogawa <i>et al.</i> , 2009
	<i>Pacifastacus leniusculus</i>	Astakine	Söderhäll <i>et al.</i> , 2005

bind a wide range of oligosaccharide (Hibino *et al.*, 2006). One of these C-type lectins called SpEchinoidin was well characterized and it was shown a possible function in the immune defense of the sea urchin because its expression is exclusively in the phagocytes after LPS-challenge (Multerer and Smith, 2004; Terwilliger *et al.*, 2004).

Molecular recognition is carried out by lectins through specific carbohydrate binding motifs. The carbohydrates exhibit several folds corresponding to different carbohydrate-binding motifs (Vijayan and Chandra, 1999). According to Vasta and colleagues (1994), C-type lectins and pentraxins play an important role in innate immune functions, since they are probably the most ancient non-self recognition/defense mechanism. Recently, a large number of C-type lectin domain-(CTLD) containing proteins has been reported in *C. elegans*, many of which show a pathogen-specific response during infection (Schulenburg *et al.*, 2008). Among the C-type lectins, mannose-binding lectins (MBL) deserve particular attention. Indeed, MBL are involved in innate immune protection and work with epithelial barriers, cellular defenses such as phagocytosis (they can act as opsonins), and pattern-recognition receptors that trigger pro-inflammatory signalling cascades. In particular, Ip and colleagues (2009) have found that MBL play a role as a co-receptor of Toll-like receptors (TLRs), since they are linked by their spatial localization on the phagosome. Furthermore, a novel involvement of MBL as a TLR co-receptor has been found, and a new paradigm for the role of these opsonins has been defined: MBL may function not only to increase microbial uptake but also to coordinate spatially, amplify, and synchronize innate immune defense mechanisms.

Chemical analysis and immunocytochemical reactions have demonstrated the presence of *N*-acetylmuramic acid (NAM) and the absence of sialic acid in the glycoconjugates in different tissue from Mollusca Gastropoda (Bolognani *et al.*, 1981; Ottaviani and Montagnani, 1989; Bolognani Fantin and Ottaviani, 1990; Ottaviani *et al.*, 1990). Accordingly, NAM has also been found in the carbohydrate fraction of a lectin present in the freshwater snail, *Planorbarius corneus* (Ottaviani and Tarugi, 1986).

Cytokines

As far as cytokines in invertebrates are concerned, several authors have reported the

presence of cytokine-like molecules in molluscs, insects, annelids, echinoderms and tunicates. Together with morphological evidence, functional experiments have also suggested the presence of invertebrate cytokines that are homologues to those in mammals. Indeed, several mammalian cytokines, e.g., IL-2, IL-8 and growth factors, stimulate cell motility, chemotaxis, phagocytosis, cytotoxicity, stress response, wound repair and the regulation of cell death in invertebrate immune cells (Ottaviani *et al.*, 2004).

Most of these findings were recorded in the 1980s and 1990s and principally concerned IL-like molecules. However, the scientific community was sceptical about the existence of invertebrate homologues of vertebrate interleukins, given the absence of experimental evidence for the presence of a real gene similarity.

Since the immune molecules, celomic cytolytic factor (CCF), from the annelid *Eisenia foetida* and human tumor necrosis factor (TNF)- α present a relevant functional similarity as a result of a shared lectin-like activity, Beschin and colleagues (2001) surmized that the evidence of invertebrate immune molecules that were hypothetically homologous to vertebrate cytokines was essentially the consequence of a functional convergence on a lectin-like activity by both the invertebrate immune factors and the vertebrate cytokines. In other words, the elusive, invertebrate cytokine-like immune factors were suggested to be lectins or, alternatively, molecules endowed with lectin-like activity, whose effects were similar to those displayed by some vertebrate cytokines (Beschin, 1999; Beschin *et al.*, 2001; Cebo *et al.*, 2002). This hypothesis was reinforced not only by the data on CCF, but also by the absence of molecular evidence (immunoblot or PCR-derived data (Beschin *et al.*, 2004) supporting the existence of invertebrate cytokines. A drawback of this analysis is, however, the extreme variability of the cytokine sequences, especially of interleukins, that makes the application of a typical sequence-based algorithm to find cytokine gene homologues almost impossible (Huisling *et al.*, 2006).

Molecular biology and functional studies have demonstrated the presence of cytokines in invertebrates: Spätzle (Morisato and Anderson, 1994) and Upd3 (Harrison *et al.*, 1998; Agaisse *et al.*, 2003) in *D. melanogaster*, Hemocyte Chemotactic Peptide (HCP) from the moth *Pseudaletia separata* (Nakatogawa *et al.*, 2009) and

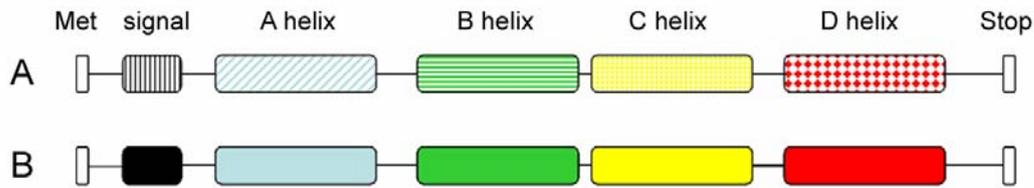


Fig. 1 Distribution of helical motifs in the preprotein form of DHF (A) and a mammalian helical cytokine (B). The name of the helices (A to D) and their position between the first Methionine (M) and the last amino acid (Stop) are given accordingly to Conklin (2004) and Conklin *et al.* (2005). The extension of the signal peptide (signal) is also reported. Boxes of similar colors (e.g., azure bars and solid azure) indicate correspondent helices with unrelated amino acid sequences.

Astakine 1 in the freshwater crayfish *Pacifastacus leniusculus* (Söderhäll *et al.*, 2005). However, there is no indication of gene homology or structure similarity between these molecules and cytokines in vertebrate species. More precisely, the conformation of Spätzle resembles that of vertebrate NGF and coagulogen in the horseshoe crab (Mizuguchi *et al.*, 1998). Upd-3 and HCP has no homology or similarity with vertebrate cytokines and immune molecules while Astakine 1 possesses a prokineticin (PK) domain found in vertebrates in molecules with many different functions, including angiogenesis and spermatogenesis (Söderhäll *et al.*, 2005). While the cited invertebrate cytokines show little or no conservations with their functional counterparts in vertebrates, signal transduction pathways appear to be well conserved. Spätzle activates Toll signalling that is considered to share significant similarity with the pathway activated by IL-1 in mammals (Lemaitre and Hoffmann, 2007), while the hemocyte-derived Upd-3 activates the JAK/STAT pathway in the fat body (Agaïsse and Perrimon, 2004).

In terms of function, the gene *spätzle* encodes for a secreted protein that requires proteolytic processing for activity (Morisato and Anderson, 1994). The protein Spätzle acts immediately upstream of the receptor Toll. *spätzle* mutant flies can recover the inducibility of *drosomycin* after injection of either recombinant full-length Spätzle or hemolymph from wild-type flies. However, the recovery of *drosomycin* induction is always subsequent to an immune challenge with mycetes or gram positive bacteria. These results demonstrate that Spätzle is a cytokine present in the hemolymph as an inactive precursor which is converted to its active form in response to infections (Ferrandon *et al.*, 2004).

upd3 has been characterized as a member of the *unpaired* (*upd*) family that activates the JAK/STAT pathway during the embryogenesis of *Drosophila* (Harrison *et al.*, 1998). Upd3 is secreted by hemocytes and subsequently activates *totA* expression in the fat body (Agaïsse and Perrimon, 2004). RNA interference data suggest that *upd-3* is not induced by activation of the Imd-pathway, or at least not by the branch controlled by the kinase dTAK1 (Malagoli *et al.*, 2008).

HCP is a chemotactic factor that displays several characteristics of a mammalian chemokine. It is a small secreted peptide, present in epidermis, granulocytes and nervous system of the larvae of the lepidopteron *P. separata*. HCP displays a strong chemotactic activity and recruits circulating hemocytes to the wound where it is supposed to enhance clotting. At present no information is available on the receptor bound by HCP and on the signalling pathway activated by this insect chemokine (Nakatogawa *et al.*, 2009).

Finally, Astakine 1 induces a strong hematopoietic response by interacting with a F₁ATP synthase receptor present exclusively on the hemopoietic tissue and not on the surface of circulating hemocytes (Lin *et al.*, 2009). A similar molecule, Astakine 2, has been identified in another crustacean, the shrimp *Penaeus monodon*, but there is still scant information on this finding (Söderhäll *et al.*, 2005).

The discovery of the above mentioned cytokines has contributed to the general acceptance of the existence of cytokines in invertebrates; however, the findings offer little help in understanding whether homologues for vertebrate interleukins can be retrieved in invertebrate models.

A significant advance in this field was the discovery of the first gene predicted to encode for a helical cytokine in *D. melanogaster* labelled *dhf* (*Drosophila helical factor*) (Malagoli *et al.*, 2007). *dhf* was recorded following the utilization of an algorithm (QT method) specifically developed to scan protein and cDNA databases and recognize sequences encoding for helical cytokines (Conklin, 2004). In vertebrates, helical cytokines represent one structural class of cytokines that include IL-2, IL-6, IL-11, IL-23, interferon α -1 and GM-CSF (granulocyte-macrophage colony-stimulating factor). As mentioned above, helical cytokines are a divergent protein family, and their phylogenetic relationship arises from the conserved protein structure, intron phases and broadly similar receptor families (Conklin *et al.*, 2005). Accordingly, no sequences similar to that of *dhf* have been retrieved in databases of other insects such as *A. gambiae* and *Apis mellifera*.

DHF (GenPept accession no. AAF53861) is a peptide of 214 amino acids, and the QT method predicts that this sequence has a helical cytokine fold with 4 core amphipathic helices (Fig. 1). Functional experiments demonstrate that DHF expression is significantly increased after immune stimulation, suggesting the involvement of this putative helical cytokine in the innate immune response of invertebrates (Malagoli *et al.*, 2007, 2008). Furthermore, using the anti-rDHF antibody, the macrophage-like *Drosophila* embryonic hemocytes (SL2 cell line) have been found to promote the secretion of DHF following exposure to heat-inactivated bacteria and after the administration of the recombinant peptide rDHF (Malagoli *et al.*, submitted). Although present data do not allow us to conclude that *dhf* is a homologue of vertebrate helical cytokines, the results do point to the first invertebrate candidate that could prove of some help in describing the evolution of one of the major classes of immune-related molecules.

Concluding remarks

The major new insight into the invertebrate immunological system suggested by the above data is that, as in vertebrates, both lectins and cytokines are involved in the chemical communication among immunocompetent cells. The functions fit into the same framework, indeed these molecules share convergent and common functions, and one of the major roles of these messenger molecules is their participation in fighting against non-self.

Even though these conclusions may appear limited, this is a synopsis of what we know about lectins and cytokines in invertebrates. These two classes of molecules have been studied with a quite different attitude in the last 25 years. Lectins have been essentially characterized from a functional and biochemical point of view. This has led to the identification of a plethora of factors, all indicated as lectins, among which it is quite difficult to find a starting point for an evolutionary analysis. Conversely, considerable attention has been paid to cytokines for purposes of molecular characterization. The continuing search for elements of conservation between invertebrate and vertebrate mediators has moved towards sequence and domain analysis as a first step, maintaining the functional characterization as a necessary but not sufficient task. As such, we can say that currently only three cytokines, i.e., Spätzle, Upd-3 and Astakine 1, are known in invertebrates. DHF is a likely further candidate, but it has still to be considered as a putative cytokine as a consequence of the sceptical attitude mentioned before. Finally, we have not referred to other cytokine-like molecules that have been found in recent years in different invertebrate taxa. In absence of the required molecular, structural and functional characterization, the respective discoverers propose these as cytokine-like molecules.

In conclusion, we would say that in the case of lectins, the adopted perspective has allowed the identification of an enormous number of family members, while the approach to cytokines has produced only three accepted members in the last

25 years. Overall and for opposite reasons, present knowledge of both lectins and cytokines must be considered inadequate for evolutionary studies.

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