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Galectins as inflammatory mediators

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Over the last decade a vast amount of reports have shown that galectin-1 and galectin-3 are important mediators of inflammation. In this review we describe how the galectins may be involved in several parts of the inflammatory process, including the recruitment of neutrophils into an infected tissue and the recognition and killing of bacteria by activation of the tissue destructive phagocytic respiratory burst. During bacterial infection or aseptic inflammatory processes, galectins are produced and released by *e.g.* infected epithelium, activated tissue-resident macrophages and endothelial cells. These extracellular galectins may facilitate binding of neutrophils to the endothelium by cross-linking carbohydrates on the respective cells. Further the galectins improve binding of the neutrophil to the extracellular matrix proteins laminin and fibronectin, and are potential chemotactic factors, inducing migration through the extracellular matrix towards the inflammatory focus. When the cells encounter bacteria, galectin-3 could function as an opsonin, cross-linking bacterial lipopolysaccharide or other carbohydrate-containing surface structures to phagocyte surface glycoconjugates. Both galectin-1 and galectin-3 have the capacity to induce a respiratory burst in neutrophils, provided that the cells have been primed by degranulation and receptor upregulation. The reactive oxygen species produced may be destructive to the invading micro-organisms as well as to the surrounding host tissue, pointing out the possible role of galectins, not only in defence toward infection, but also in inflammatory-induced tissue destruction.
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Introduction

Galectins are a growing family of β-galactoside-binding animal lectins [1]. At least twelve galectins are present in humans, and altogether the family today comprise fourteen members [2,3]. The galectins are present intracellularly in the cell cytosol as well as in the nucleus. Despite the fact that they lack a signal peptide they can be secreted extracellularly by a so far unknown pathway, similar to that utilised by IL-1 and annexin I [4]. The wide distribution of the galectins, both with regard to species and tissues, suggests that they exert many different functions, which have also been reported in a great number of studies over the last decade. The two most extensively studied galectins are galectin-1 and galectin-3. Galectin-1 belongs to the prototype galectins, consisting of two identical carbohydrate recognition domains (CRDs) that may aggregate into homodimers. Galectin-3 is a chimeric galectin, containing one CRD linked by a collagenase-sensitive domain to an N-terminal aggregating domain that enables the molecule to form homodimers or hexamers. The evolving knowledge about the presence of galectin-1 and galectin-3 in epithelium, endothelium and activated tissue macrophages, cells that are central with regard to inflammation, has inspired us and others to investigate the involvement of galectins in inflammatory processes.

The inflammatory process can at the macroscopical level be defined by its resulting symptoms, *i.e.*, what is known as the cardinal signs: rubor (redness), calor (temperature), tumor (swelling), dolor (pain) and functio laesa (loss of function) [5]. On a microscopical level, the process leading to these symptoms, is a complex network involving vascular dilation with increased permeability and blood flow, exudation of fluids and plasma proteins and leukocyte migration into the inflammatory focus. The inflammatory process can be initiated *e.g.* by infectious components, trauma, or autoimmune activities, and is enhanced by activation of the complement cascade, the fibrinolytic system, the kinin-forming system, and the coagulation pathway. This results in production of various inflammatory mediators. As a result, phagocytic cells (monocytes/macrophages and neutrophils) infiltrate the inflamed tissue and proinflammatory and antibacterial effector functions are activated, ultimately killing the invading bacteria. In severe inflammation, the antibacterial effector functions may contribute to the tissue destruction.

The involvement of lectin—carbohydrate interactions in the inflammatory process has during the last decade become more

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and more evident [6–8]. The initial binding of leukocytes to the endothelium is mediated by selectins, C-type lectins that bind primarily to sialylated *Lewis antigens* on the endothelium or vice versa. The trafficking of leukocytes towards an inflammatory focus may also be directly influenced or guided by lectin—carbohydrate interactions as illustrated by the fact that the lung surfactants, which are collectins belonging to the C-type family of lectins, have been shown to possess chemotactic activity [9]. By binding to non-host carbohydrate structures present on the surface of various microorganisms, including bacteria, fungi, parasitic protozoa and viruses, the collectins can also promote complement activation or function as opsonins, promoting phagocytic uptake of the coated bacteria [10].

We hope to convince the readers of this review that galectins, and in particular galectin-1 and galectin-3, take position as important inflammatory regulators among other endogenous mediators such as interleukins and leukotrienes. Further, we show that the interaction of polymorphonuclear granulocytes (neutrophils) with galectin-1 and galectin-3 may be of importance for both the antibacterial and proinflammatory effects of these cells, and that these activities are finely tuned by priming mechanisms to ascertain activation only at the proper time and place.

Role of galectins in leukocyte recruitment

Increased adherence of circulating leukocytes to the endothelium initiates the recruitment of neutrophils from the blood stream into an infected tissue. Upregulation of selectins on both neutrophils and endothelium results in weak cell-cell interactions, slowing down the speed of the circulating neutrophil, and allowing it to roll on the endothelium. The rolling process is an initiation signal for a more firm, integrin-mediated adhesion that arrests the cell at the endothelial surface and allows it to flatten out. According to the current paradigm, the firm adhesion will not occur without being preceded by the selectin-mediated rolling.

The selectin/integrin paradigm is however not always followed, as illustrated by the scenario during an infection with *Streptocuccus pneumonia* [11,12]. At the onset of such an infection (but not during an *Escherichia coli*-induced infection), resident alveolar macrophages produce and secrete galectin-3, and the amount of galectin-3 released correlates to the amount of transmigrated neutrophils [13]. This suggests that galectin-3 has a direct function in mediating the adhesion of neutrophils to the endothelium and induce extravasation into the lung [13]. The fact that cellular cross-linking is dependent on the N-terminal aggregating domain of the galectin illustrates the importance also of the non-carbohydrate-binding domain of galectin-3.

Leukocyte recruitment has also been studied in galectin-3 null mutant mice. These animals show no morphological abnormalities and have no apparent phenotypes under standard laboratory conditions [14]. They are, however, deficient with respect to the recruitment of phagocytic cells in response to a non-infectious inflammatory agent [15,16]. The reduction in the number of granulocytes in the peritoneal cavity is evident only in the late stage (after 4 days) of inflammation [16]. The initial influx (day 1) was however not affected, suggesting that two different recruitment pathways are functional and that galectin-3 is of crucial importance for the latter part and/or for the maintenance of neutrophils into the tissue. It has however, to be taken into account that galectin-3 functions could be compensated for by other galectins with similar carbohydrate specificity. Investigation of the galectin-1 null mutant mice [17] in an inflammatory setting has still to be performed.

Galectin-promoted adhesion to the endothelium

It is an appealing idea that galectins produced by circulating blood cells or endothelial cells could induce leukocyte attachment to the endothelium by cross-linking carbohydrates on the respective cell surfaces, thereby mediating adhesion *e.g.* when the integrin pathway is insufficient. Human endothelial cells do in fact express galectin-1, even though the basal levels are very low [18,19]. When stimulated with minimally oxidised low density lipoprotein (MM-LDL) the expression increases, and galectin-1 is thought to be secreted from the activated cells [19]. A similar increase in expression can also be achieved by activating the cells with a combination of chemokines and bacterial lipopolysaccharides (LPS).

Whether circulating leukocytes also secrete galectins to participate in the interaction with the endothelium is more uncertain. An increased expression of galectin-3 mRNA has been demonstrated in neutrophils isolated from patients suffering from parasite infections, allergy, and eosinophil disorders [20], but whether the protein is secreted is not known. Monocytes, however, can release vast amounts of galectin-3, *e.g.* upon stimulation with bacterial LPS [21]. Whether such production could provide a source of galectin-3 also in circulation can only be speculated upon. In addition, galectin-3 has been shown to induce production of IL-1 in macrophages [22]. IL-1 is involved in a variety of immunological processes, and is known to induce leukocyte adhesion molecules (ICAM, ELAM and VCAM) on vascular endothelium. Hence, in this more indirect way, galectin-3 could act as an adhesion promotor.

Taken together, these findings show that the prerequisites for galectin-promoted adhesion of the neutrophils to the endothelium are present. However, much more evidence has to be presented in the field before any definite conclusions regarding the galectin-involvement can be drawn.

Galectin-induced transmigration and chemotaxis

Following adhesion, leukocytes migrate across the endothelial lining of the micro-vessels by a process called diapedesis. They then start to move towards the inflammatory focus through the extracellular matrix (ECM) by chemotaxis along a gradient of chemoattractants, derived from either endogenous cells or invading microbes. Some galectins possess chemotactic capacity. In addition to galectin-9 (described in detail elsewhere in this

issue), shown to be chemotactic for eosinophils, galectin-3 is the only galectin so far shown to possess chemotactic activity for inflammatory cells.

Galectin-3 is chemotactic for human monocytes and macrophages, and it is much more potent than the classical chemokine monocyte chemoattractant protein-1 (MCP-1 [23]). By desensitisation experiments, Sano *et al.* have shown that galectin-3 uses a receptor different from the currently known chemokine receptors on monocytes [23]. Macrophages lack the receptor for MCP-1, and therefore do not respond to the classical chemokine, but respond readily to galectin-3. As in many other galectin-3-induced processes, both the CRD and the N-terminal domain are required for the chemotactic capacity.

In contrast to galectin-3, no chemotactic capacity has been reported for galectin-1. In fact, in PLA_2-induced rat hind paw oedema, transmigration of neutrophils as well as mast cells into the tissue was reduced in the presence of galectin-1 [24]. Galectin-1 has also been shown to inhibit eosinophil migration in nasal polyps [25], suggesting that the signals generated by galectin-1 binding inhibit rather than promote migration of inflammatory cells.

The movement of the neutrophil across the ECM requires not only the presence of a chemoattractant but also adhesion to the underlying protein/carbohydrate matrix. The involvement of galectins in adhesion to the ECM is well established. Both galectin-1 and galectin-3 have been shown to promote binding between the ECM protein laminin and a variety of cells. Galectin-1 molecules binding to laminin promote cell adhesion of Chinese hamster ovary cells [26] and human melanoma cells [27]. This adhesion-promoting property is cell specific, and the adhesion of other cell types to laminin is in fact inhibited by galectin-1 [28] and galectin-3 [29], as in the case of T-cell adhesion to the ECM [30]. Fibronectin, another ECM protein, is also a ligand for galectin-1 [31], but concerning neutrophils, the possible role for galectin-1 as a link to the ECM has not yet been investigated.

Galectin-3 can promote the adhesion of neutrophils [32] as well as other cell types [33,34] to laminin in an integrin-independent manner. This bridging by galectin-3 of neutrophils to laminin appears to involve activation of the cells via Ca^{2+} and Mg^{2+} dependent processes. At high concentrations of galectin-3 (>50 μg/ml) the lectin also induces adhesion to fibronectin [32]. However, this is completely integrin-dependent as shown by its inhibition by β_2-integrin antibodies.

Galectins in phagocytosis and killing of microbes

At the site of infection the primary function of the neutrophils is to eliminate the invading micro-organisms by phagocytosis and subsequent intracellular killing. The phagocytic process involves both attachment of the neutrophil to the bacterium and subsequent engulfment. Inside the phagosome, the bacterium encounters two different killing strategies of the neutrophil, the oxygen-independent, relying on the release of bactericidal agents from the granules into the phagosome, and the oxygen-dependent in which highly destructive oxygen metabolites are produced by the NADPH-oxidase and secreted into the phagosome.

Bridging role of galectins in phagocyte—bacterial interaction

An interaction between a phagocyte and a bacterium can be facilitated by opsonisation with antibodies or complement factors binding to the Fc-receptors or complement receptors, respectively. Direct lectin-carbohydrate interactions are also of importance for the phagocytosis of bacteria particularly at sites where plasma proteins (including antibodies and complement) are scarce [35], *e.g.* in the urinary tract. Galectins are potential opsonins, having the capacity to cross-link two or more cells by binding to cell surface carbohydrates. Indeed, galectin-3 can bind lipooligosaccharides of certain non-enteric gram negative bacteria (*N. gonorrhoeae* and *H. influenzae*) [36], and cross link them to neutrophils [37,38]. Moreover, we have preliminary data showing that the phagocytosis-induced respiratory burst in neutrophils is influenced by the presence of galectin-3 (unpublished data).

The interaction of galectin-3 with LPS of gram-negative bacteria has been investigated and shown to involve two distinct binding sites, allowing association with rough or smooth LPS through two independent ways of recognition [39]. The interaction between galectin-3 and smooth LPS from *Klebsiella pneumoniae* having a β-galactoside-containing polysaccharide chain is mediated by the galectin CRD, in a lactose sensitive manner. Contrarily, rough LPS from *Salmonella minnesota* R7 (Rd mutant), devoid of β-galactosides, is recognised by the N-terminal domain of galectin-3 binding to the lipidA/inner core of LPS. This interaction is lactose-resistant, but can be inhibited by the N-terminal-recognising antibody M3/38. These data indicate that galectins can function as pattern recognition molecules, not only binding to the variable carbohydrate moieties of bacterial LPS but also by having the capacity to bind the more conserved inner core.

Recently a role of galectin-3 in phagocytosis was indicated by studies of macrophages from null mutant mice, which showed a slower phagocytosis than wild type macrophages [40]. The mechanism was indicated to involve intracellular rather than extracellular galectin-3.

Galectin-induced activation of the neutrophil respiratory burst

The neutrophil NADPH-oxidase is an electron-transporting enzyme that reduces oxygen to superoxide anions upon activation. In its inactive form it is composed of four cytosolic components and a membrane-bound heterodimeric b-cytochrome. Upon activation of the neutrophil the cytosolic components translocate to the membrane, where they associate with the b-cytochrome, assembling into the active NADPH-oxidase [41]. The b-cytochrome is situated both in the plasma membrane and in the membrane of the specific granules. Depending

on which oxidase pool that is activated, superoxide anions are either released extracellularly or produced at an intracellular site, [42,43].

Both galectin-1 and galectin-3 are able to activate the NADPH-oxidase of human neutrophils in a lactose-sensitive manner, but only under certain circumstances (see below) [44–46]. For galectin-3, the activity is dependent not only on the CRD but also on the N-terminal, aggregating domain as the collagenase-treated molecule (consisting only of the CRD) is not sufficient to induce an oxidative burst [45]. This is supported also by the fact that the monoclonal antibody B2C10, binding to the N-terminal domain of galectin-3, has inhibitory effects on the galectin-3 induced NADPH-oxidase activity [47]. Whether the N-terminal domain is essential due to its cross-linking capacity has still to be investigated. However, since galectin-3 shares the capacity to cross-link receptors with the equally potent galectin-1 (which dimerises and thus has two CRDs), it is reasonable to believe that this feature is of importance to the activating potential.

Priming through granule mobilisation as a prerequisite for neutrophil activation by galectins

Galectin-1 and galectin-3 have been shown to induce an oxidative burst in neutrophils that have extravasated into the tissue, but not in peripheral blood neutrophils [44,45]. Such enhancement of cellular activity in exudated neutrophils is referred to as priming and occurs not only in response to galectins, but also to various inflammatory mediators, chemoattractants and other ligands [48–50]. Many priming mechanisms have been suggested, *e.g*. calcium elevations, protein phosphorylations and combinations of these two [51,52]. It has also been shown that intracellular organelles are mobilised to the cell surface during extravasation [53], resulting in an increased exposure of various receptors, which could be responsible for the increased response to *e.g*. galectin-3. Human neutrophils contain at least four such organelles: the azurophil granules, serving primarily an intracellular role, and the specific granules, the gelatinase granules and the secretory vesicles, functioning as secretory organelles (for a review see [54]). During *in vivo* exudation, these secretory organelles are mobilised to various extents; the secretory vesicles are completely mobilised together with 40% of the gelatinase granules and 20% of the specific granules [53]. To investigate whether priming of the galectin-3 response was due to receptor upregulation, we used *in vitro* priming protocols, pre-treating the cells with the chemotactic peptide fMLF or bacterial LPS, respectively [44,45,55]. By correlating the release of soluble granule proteins as well as the exposure of new proteins on the plasma membrane to the degree of responsiveness, the ability of *in vivo* (exudated cells) as well as *in vitro* (by fMLF or LPS) primed neutrophils to respond to galectin-1 and galectin-3 was shown to parallel the mobilisation state in the cells [45,55]. Although correlative, our findings suggest that the galectin-3-binding receptors reside in the gelatinase and possibly also the specific granules in unperturbed (resting) cells while the receptors for galectin-1 appears to be localised in the secretory vesicles and gelatinase granules, based on the fact that it is somewhat easier to prime the neutrophils for galectin-1 activation [44].

We hypothesise that the regulation of receptor exposure is of crucial importance for modulation of the neutrophil responsiveness. Activation of the NADPH-oxidase and release of toxic oxidative metabolites must be limited in time and place to where it is required, *i.e*. at the inflammatory focus. If the neutrophils were sensitive to activating galectins already in the bloodstream, endothelial galectin production in the vasculature would be directly harmful. In fact, we have preliminary data showing that peripheral blood neutrophils from patients suffering from severe infections are primed and respond to galectin stimulation (unpublished data). How a systemic neutrophil response to galectins influences the outcome of such disease states has to be clarified.

Neutrophil galectin receptors

Both soluble and membrane-bound galectin-3-binding molecules have been isolated and characterised, but very few studies have been able to verify the involvement of one specific molecule in cell activation by galectin-3. In the T lymphoblastoid Jurkat cells, galectin-3 binds CD98, triggering a Ca^{2+} influx [56]. This galectin-3-binding protein is also present in macrophages and has been isolated from these cells on a galectin-3 affinity column together with the α-subunit (CD11b) of CR3, and the lysosome-associated membrane glycoproteins (Lamp)-1 and -2 [57]. Circumstantial evidence indicates that the Lamps are responsible for galectin-3 binding to the human melanoma cell line A375 [58]. In addition, these cells secrete soluble galectin-3-binding glycoproteins including the Mac-2 binding protein [59,60]. In colon carcinoma cells, the galectin-3-interacting proteins include members of the CEACAM family as well as the Lamps [61].

Upon detecting the neutrophil-activating capacity of galectin-3, Yamaoka and co-workers affinity purified galectin-3-binding proteins from neutrophil plasma membrane and showed the presence of proteins belonging to the CEACAM family [46]. We later confirmed this finding and showed that galectin-3 binds two family members, CEACAM1 and CEACAM8, as well as Lamps-1 and -2 [62]. By investigating the presence of these proteins in non-responding HL-60 cells, the CEACAMs were concluded to be the most probable receptor candidates. As the CEACAMs are localised in the neutrophil gelatinase and specific granules, they also fit our granule mobilisation data, *i.e*. that the galectin-3 receptors should be stored intracellularly in resting cells and upregulated primarily from the gelatinase and specific granules during priming (see above).

Concerning the receptors for galectin-1, the picture is more uncertain. Avni *et al.* [63] showed that galectin-1 co-immunoprecipitates with CR3 (CD11b/CD18) on murine bone marrow-derived macrophages in a lactose sensitive manner. The two proteins also co-localised as shown by fluorescence

microscopy, indicating that CR3 might be a galectin-1 receptor. The authors speculate that the binding of galectin-1 to CR3 can modify the binding capacity of CR3 to its ligand, thus regulating the cellular response. We have earlier shown that the galectin-1 activating potential correlates with CR3 upregulation on the neutrophil cell surface [44]. However, when allowing galectin-1 to bind neutrophil proteins on Western blots, several proteins bound galectin-1, thus making it difficult to point out one specific receptor. It should also be noted that whereas galectin-1 activates both the plasma membrane and the specific granule pool of the NADPH-oxidase, other CR3 agonists activate only the granule localised oxidase [64].

Galectin-1 and galectin-3 both induce an oxidative burst with similar kinetics under similar circumstances (*i.e.* priming via degranulation [44,55]). It would thus be reasonable to believe that the two galectins would activate the neutrophil via the same β-galactoside receptor. Talking against this is the fact that the receptor/s for galectin-1 (as stated above) are most probably subcellularly localised to the secretory vesicles and the gelatinase granules [44], whereas the receptors for galectin-3 seem to be present in the gelatinase- and specific granules [45]. Another indication that different receptors are responsible for the galectin-1 and galectin-3 responses is the fact that the two galectins have different concentration dependencies. At 640 μg/ml, galectin-1 has not reached a maximal activating concentration, whereas galectin-3 has its maximal activation potential at 40 μg/ml for the plasma membrane bound NADPH-oxidase and as low as 20 μg/ml for the intracellular NADPH-oxidase [44].

Concluding remarks

The research field of innate immunity has gained much attention in recent years and is rapidly expanding in parallel to the intensely developing field of glycobiology. In the overlap between these areas one can foresee the unfolding of many new concepts and ideas pertaining not only to the basic functions of lectins and carbohydrates in innate immunity and inflammation, but as an extension also to development of new therapeutic strategies for treatment and prophylaxis of inflammatory diseases. In this review, we have attempted to give an overview of the present knowledge on galectin participation in the inflammatory process (Figure 1). It is evident that the galectins may

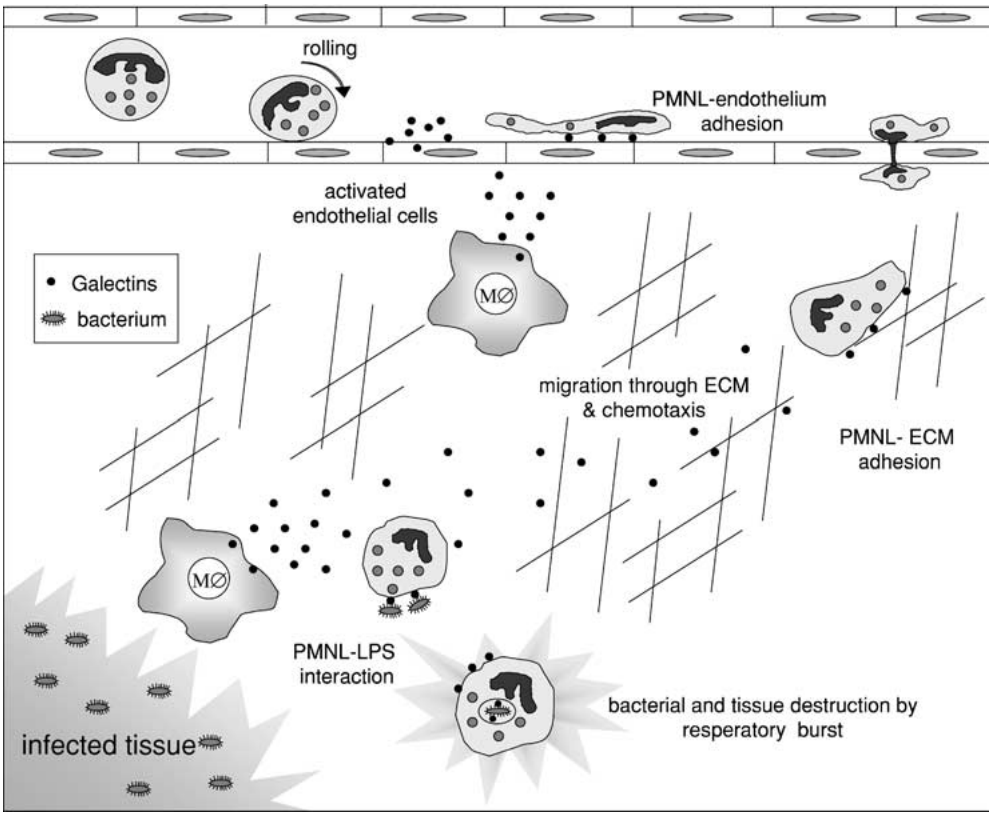

Figure 1. Schematic overview of the involvement of galectins in the inflammatory process. Galectins (black dots), produced by *e.g.* macrophages (Mø) and endothelial cells may be involved in the recruitment of polymorphonuclear leukocytes (PMNL) from the blood stream. The galectins may cross-link the PMNL with the endothelium, as well as facilitating the migration through the extracellular matrix (ECM) by being chemotactic and cross-linking the cell to the underlying protein matrix. Further, in the interaction with invading bacteria, galectins may function as opsonins, facilitating the attachment between the PMNL and bacteria. By activating the respiratory burst in the PMNL, galectins may participate in both the antibacterial defence as well as in the inflammation-associated tissue destruction.

function as both pro- and anti-inflammatory mediators and it is of outmost importance that we increase our understanding of this balance conferred by the galectins. This may then provide the means to define possible points of attack for regulating the inflammatory process *in vivo*.

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