Galectins and microenvironmental niches during hematopoiesis

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Purpose of review

Galectins, a family of evolutionarily conserved glycan-binding proteins, are involved in the regulation of multiple cellular processes (e.g. immunity, apoptosis, cellular signaling, development, angiogenesis and cellular growth) and diseases (e.g. chronic inflammation, autoimmunity, cancer, infection). We discuss here how galectins contribute to the development of specialized microenvironmental niches during hematopoiesis.

Recent findings

An expanding set of data strengthens a role of galectins in hematopoietic differentiation, particularly by setting specific interactions between hematopoietic and stromal cells: galectin-5 is found in reticulocytes and erythroblastic islands suggesting a major role during erythropoiesis; galectin-1 and 3 are involved in thymocyte apoptosis, signaling and intrathymic migration; galectin-1 plays critical roles in pre-BII cells development. Moreover, expression of galectins-1 and 10 are differentially expressed during T-regulatory cell development. Various galectins (3, 4, 5, 9) have been reported to be regulated during myelopoiesis and traffic into intracellular compartments, dictating the cellular distribution of specific glycoproteins and glycosphingolipids.

Summary

The abundance of galectins in both extracellular and intracellular compartments, their multifunctional properties and ability to form supramolecular signaling complexes with specific glycoconjugates, make these glycan-binding proteins excellent candidates to mediate interactions between hematopoietic cells and the stromal microenvironment. Their secretion by one of the cellular partners can modulate adhesive properties by cross-linking specific glycoconjugates present on stromal or hematopoietic cells, by favoring the formation of synapses or by creating glycoprotein lattices on the surface of different cell types. Their divergent specificities and affinities for various glycoproteins contribute to the multiplicity of their cellular interactions.

Keywords

galectins, hematopoiesis, microenvironmental niches

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Introduction

In the bone marrow, hematopoiesis occurs within a hematopoietic cell compartment located in the extravascular spaces between the sinuses. Three-dimensional images have recently been obtained by confocal microscopy showing the complex architecture of the bone marrow [1]. Interactions between hematopoietic cells and (nonhematopoietic) stromal cells are essential for the development and differentiation of certain lineages by supplying various factors. Different stromal cells can create such microenvironmental niches upon which specific hematopoietic cells could reside or pass in transit, and mature. For example, hematopoietic stem cells (HSCs) are localized closed to the endosteum of the bone marrow and around blood vessels, interacting with osteoblasts and endothelial cells that could define osteoblastic and vascular niches, respectively [2]. Erythroblastic islands consist of a central macrophage extending cytoplasmic protrusions to a ring of surrounding erythroblasts. The macrophage functions as a nursing cell for the proliferation and differentiation of erythroid cells, and phagocytoses the extruded nuclei at the end of erythroid differentiation. In the case of B-cell development, the spatial organization of the microenvironmental niches is less clear, since the B cells seem to associate with distinct cytokine-secreting cells in a spatio-temporal regulated fashion before they enter the vascular sinuses. Interestingly, it has been recently shown that the bone marrow microenvironment is tightly regulated *in vivo*, as erythropoietin treatment causes bone remodeling with an altered B lymphopoiesis to the benefit of erythropoiesis [3]. T cells originate in the bone marrow but uniquely complete their development in the specialized environment of the thymus. Only a fraction of thymocytes are selected to survive, migrating out of the thymus as naïve mature T

lymphocytes. Effective interactions between developing thymocytes with thymic epithelial cells, dendritic cells and macrophages are critical for the establishment of a fully competent T-cell compartment.

The galectin family

Galectins are a family of soluble lectins that bind β-galactoside-containing glycans in a calcium-independent way and share characteristic amino acid sequences defining conserved carbohydrate recognition domains (CRDs), common structural folds and pleiotropic regulated expression in several tissues [4]. Although all CRDs bind to a common disaccharide (Galβ1–4-NAcGlc; LacNAc), each galectin has an individual carbohydrate-binding preference due to variabilities in the CRD sequence [5]. To date, 15 mammalian galectins have been identified in diverse tissues and species, which can be subdivided into those that have one CRD (prototype: galectins-1, 2, 5, 7, 10, 11, 13, 14 and 15), those that have two different CRDs (tandem-repeat type: galectins-4, 6, 8, 9 and 12), and galectin-3 containing one CRD connected to an N-terminal aggregating domain that enables the molecule to form pentamers (chimera-type) [6]. Some prototype galectins can form dimers through noncovalent interactions and this dimerization is critical for cross-linking glycoconjugates, transducing intracellular signals and forming multivalent lectin–glycan clusters called 'lattices' on the surface of target cells. Whereas some galectins such as galectin-1, 3 and 9 are broadly expressed [7,8], other members of the family such as galectin-10 and 12 are preferentially expressed in certain tissues [9]. Galectins are expressed at different extents in nonhematopoietic tissues contributing to embryogenesis, connective and neural tissues development, as well as muscle cell differentiation and vasculogenesis [10]. Notably, they are abundantly expressed in hematopoietic tissues, in which they play critical roles in mediating cell communication and signaling by bridging hematopoietic and nonhematopoietic compartments.

Galectins have typical features of cytosolic proteins, yet some of them are secreted by cells even though they do not possess a signal sequence required for protein secretion through the classical secretory pathway. Although the molecular bases of their extracellular release are still poorly understood [11], these lectins can be found in the extracellular space and bound onto the plasma membrane. Among naturally occurring glycoconjugates, glycoproteins that contain poly-*N*-acetyllactosamines are especially good ligands for galectins, with specificities that can vary with the degree of *N*-glycan branching and the presence of sialic acid or fucose on the chain [5,12]. Their extracellular functions are somehow related to their abilities to cross-link glycoproteins or glycolipids on the cell surface [13]. Galectins can bind glycoconjugates present onto the same membrane leading to the formation of galectin–glycan lattices, affecting endocytosis and delivery of intracellular signals [14]. A variety of glyco-receptors have been proposed to serve as possible receptors for galectins. These include CD45, CD43, CD7, T-cell receptor and GM1 on developing and peripheral T cells [15–17], α4β1 (VLA-4), α5β1 (VLA-5), and α4β7 integrins as well as the B-cell receptor (BCR) on developing B cells and bone marrow stromal cells [18], CD43 on dendritic cells [19,20] and neuropilin-1 on endothelial cells [21]. However, the role of galectins is not restricted to their extracellular effects; galectins also function intracellularly by interfering with specific signaling pathways [9]. Within hematopoietic niches, galectin–glycan interactions control communication between hematopoietic and nonhematopoietic cells, modulate cellular trafficking and deliver intracellular signals required for migration and survival. This review aims to integrate scattered data on galectins within different hematopoietic compartments and to discuss future directions and implications for this emerging field.

Key points

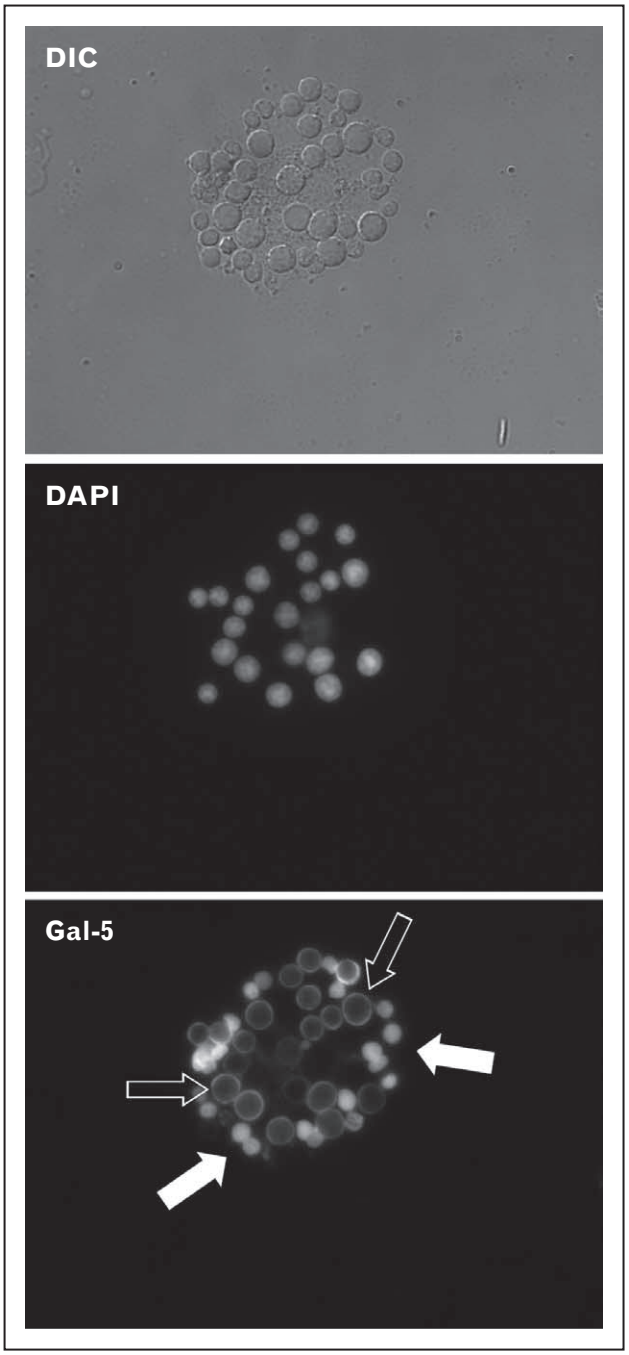
- Various galectins are expressed and secreted by hematopoietic or stromal cells, and then bind to β-galactoside-containing glycoconjugates present on the cell surface.
- Specific glyco-receptors on the surface of the different hematopoietic cell lineages interact with the distinct galectins.
- Within hematopoietic niches, galectin–glycan interactions control intercellular communication, modulate cellular trafficking and induce intracellular signals.

Role of galectins in erythropoiesis

Erythroblastic islands, first characterized by Marcel Bessis [22], are distinct anatomic units formed by a central macrophage associated with differentiating erythroblasts [23,24]. The central macrophage provides the growth-promoting and differentiation-inducing molecules which are necessary for erythroblast differentiation. This requires intimate cell contact between erythroblasts and macrophages [25]. Various adhesion molecules have been demonstrated to be involved in the association of erythroblasts with central macrophages. Among them, erythroblast macrophage protein (Emp) is a molecule identified in both erythroblasts and macrophage membranes [26], whereas the integrin α4β1 present on the erythroblasts interacts with vascular cell adhesion molecule 1 on the central macrophage [27]. More recently, erythroid intercellular adhesion molecule-4 (ICAM-4)

has been described to bind to macrophage α_V integrin [27]. Apart from these well characterized partners, other receptors on macrophages were described, including lectin-like sheep receptor [28], now termed sialoadhesin, which binds erythroblast sialylated glycoproteins. Another lectin-mediated interaction was reported in erythroblast islands, involving a β-galactoside-specific lectin purified from rabbit bone marrow and called erythroid developmental agglutinin (EDA) [29]. Indirect immunofluorescence studies showed that the extracellular lectin was associated with the erythroblast cell surface [30]. Similarly, a soluble β-galactoside-binding lectin initially purified from extracts of rat lung and referred to as RL18 [31] was then localized to red cells and designated as galectin-5 [32]. Galectin-5 is specifically expressed in rat and shows extensive identity with the C-terminal part of galectin-9 [33], likely due to gene duplication and ensuing sequence divergence [34]. Very interestingly, galectin-5 can be localized surrounding erythroblasts associated with the central macrophage of rat erythroblastic islands (Fig. 1), suggesting, like EDA, the involvement of galectin-5 in cell–cell communication. It is still unknown whether galectin-5 is engaged in trans-interactions or whether it could create lattices with glycosylated integrins or other cell adhesion molecules on the erythroblast surface. Such a localization of galactoside-binding lectins in erythroblastic islands of species other than rabbit and rat has not been reported, even though the erythroleukemic cell line K562 was shown to secrete galectin-1 when induced to differentiate [35]. This is likely related to differences in tissue sialylation patterns among species (e.g. relative lack of trisialyl and tetrasialyl-*N*-glycans in rat, differences in sialic acid types found on erythrocytes of various mammalian species) [36]. However, the fact that EDA and galectin-5 are expressed by the erythroid cells does not preclude the possibility that galectins secreted by the stromal macrophage might be involved in a similar process. Of note, the Forssman glycosphingolipid, specifically present on the surface of the mouse central macrophage and not on other stromal cells, possesses GalNAcα1–3GalNAcβ1–3Galα1–4Galβ1–4Glcβ1-ceramide structure potentially recognized by galectins. Moreover, expression of Forssman antigen on the cell surface has been shown to be regulated during the macrophage maturation [37], suggesting a functional role at the nurse cell stage. The other role of macrophage is to engulf and degrade the extruded nucleus at the end of terminal differentiation. Various components of the membrane surrounding the nucleus were shown to contribute to its phagocytosis. Phosphatidylserine exposure has been demonstrated *in vitro* to participate in the process [38]. At this point, it is interesting to note that TIM-3, a receptor highly specialized for recognition of phosphatidylserine, can interact with galectin-9 in a nonexclusive manner [39,40]. In this regard, Cummings *et al.* demonstrated that some members of the galectin family, including galectin-1, 3, 4 and 8, are specialized in promoting phosphatidylserine exposure in different cell types and to prepare cells for phagocytic removal through

Figure 1 Galectin-5 in rat erythroblastic islands



Erythroblastic islands from rat bone marrow were prepared as in [28], labeled using galectin-5 rabbit antiserum and donkey antirabbit IgG-Alexa 488 (Invitrogen), and mounted using 4′,6-diamidino-2-phenylindole (DAPI)-containing ProLong antifade reagent (Molecular Probes). Observation was carried out using a Zeiss Axioimager and a Zeiss 63x Plan-Apo 1.4 oil objective. Note the galectin-5 surrounding the erythroblast nuclei (black arrows) and labeling the anucleated reticulocytes (white arrows).

a mechanism called 'preaparesis' [41,42]. These data strongly suggest that galectins may be involved in end terminal processes of erythroid differentiation involving phosphatidylserine exposure and macrophage engulfment. Moreover, adhesive proteins such as Emp and β1 integrin partition predominantly to the plasma membrane surrounding the nucleus, whereas glycophorin A is mainly sorted to nascent reticulocytes [43]. It was suggested that the connectivity with the spectrin-based skeleton is involved in this sorting process, which was confirmed by demonstrating the abnormal distribution of skeleton-associated proteins to the nucleus in cells presenting protein 4.1 or ankyrin-1 deficiencies [44•]. A nonconflicting hypothesis is that the proteins not connected with the cytoskeleton could be endocytosed and directed towards the nucleus extrusion site. As recently reported, coalescence and fusion of the vesicles would allow the membrane supply necessary for nucleus extrusion [45••] and could also favor membrane protein sorting around the nucleus. One possibility is that galectins could be involved in this sorting process, which would also apply to the sorting of desialylated glycoproteins towards the extruded nucleus [46,47]. Finally, macrophage intracellular galectin-3 might favor the phagocytosis of the extruded nucleus as described for apoptotic cells [48].

Erythroid cells proliferation and differentiation into nonnucleated reticulocytes occurs on erythroblastic islands. Discrimination of the successive differentiating stages has been recently yielded by analysis of CD44 expression on erythroblasts [49]. Reticulocytes are then produced upon nucleus extrusion from orthochromatic erythroblasts in the bone marrow. Their maturation lasts 2–3 days, first in the bone marrow and then in the blood circulation. During this maturation process, reticulocytes lose their intracellular organelles, such as mitochondria, eliminated through selective autophagy [50]. Reticulocytes also undergo extensive membrane remodeling [51]. Part of this remodeling processes is carried out in peripheral reticulocytes by selective elimination of membrane proteins [e.g. transferrin receptor (TfR)] sorted into multivesicular endosomes (MVEs) and released through the exosomal pathway [52,53]. Note, however, that similar TfR-enriched MVEs were described in differentiating erythroblasts from fetal rat liver [54], allowing the hypothesis of exosome secretion at earlier differentiation stages than the reticulocyte. We recently reported that galectin-5 is present on the surface of exosomes released by peripheral rat reticulocytes [55•], and suggested that it could be involved in removal of specific glycoproteins (e.g. Lamp2). In agreement with a lower affinity of galectin-5 [56] and other galectins [12] for sialylated proteins, using an inhibitor of sialidase during in-vitro maturation of reticulocytes, we found that desialylated Lamp2 was preferentially eliminated through exosomes (unpublished data).

Galectin-5 was found in the endosomal lumen of rat reticulocytes and thus could access the ectodomain of glycoproteins, even though the membrane translocation site is still unknown [55•]. Of note, galectin-based sorting of distinct glycoproteins with complex-type *N*-glycans such as dipeptidyl peptidase IV, carcinoembryonic antigen, or mucin-like membrane mucin 1 (MUC1) through the endocytic/recycling pathway has recently been described for galectin-4 in the apical biosynthetic pathway in enterocyte-like cells [57]. Similarly, trafficking of galectin-3 through endosomal organelles of polarized and nonpolarized cells has recently been reported [58], together with a regulation of MUC1 and epidermal growth factor receptor cellular distribution in pancreatic cancer cells by galectin-3 [59•], and a control of apical-basal polarity in Madin–Darby canine kidney cells by galectin-9 interaction with Forssman glycosphingolipid [60••].

One could imagine that galectins that are bound exosomes are involved in signal transmission from erythroid cells to neighboring cells. This could involve signaling due to galectin binding to a cell surface receptor, as shown recently for galectin-9-associated exosomes secreted by nasopharyngeal carcinoma cells infected by Epstein–Barr virus which associate with TIM-3 present on T lymphocytes [61]. On the contrary, galectin-bound exosomes could be internalized by recipient cells [55•] and could provide effector molecules such as microRNAs contained inside the vesicles, a process that may be well suited for controlling space-confined processes [62,63].

Role of galectins in B and T lymphopoiesis

B lymphocytes differentiate in the bone marrow through successive developmental stages characterized by the expression of cell surface markers and sequential rearrangement of genes coding for immunoglobulin chains that constitute the BCR [64]. Cells expressing the pre-BCR differentiate into the pre-BII stage and start to proliferate. At this stage, pre-BCR is able to generate signals for B-cell development through engagement by unconventional ligand – a process termed tonic signaling [65]. Galectin-1 secreted by stromal cells was identified as such a ligand, interacting with surrogate light chain of pre-BCR by direct protein–protein interaction [66]. At the same time, CRDs of galectin-1 dimers can bind glycoproteins presenting galactoside-containing determinants on the surface of both pre-B cells and stromal cells, inducing the formation of a synapse at the contact zone between the two cell types. As mentioned above, integrins (α4β1, α5β1 and α4β7) expressed by pre-B cells were identified as major counter-receptors of galectin-1. Clustering of pre-B-cell integrins was shown to induce galectin-1-dependent pre-BCR relocalization and signal transduction *in vitro* [18], which was confirmed *in vivo* using galectin-1-deficient mice [67]. The authors recently

extended their observations, characterizing the stromal cells secreting galectin-1 as a cell population distinct from IL-7^+ stromal cells [68••], contributing to the understanding of the migratory route of B-cell development.

Once in the periphery, B lymphocytes are also exposed to galectins present in the spleen, lymph nodes and peripheral tissues. In fact, galectin-1 is up-regulated by activation signals [69] and contributes to the differentiation of germinal center B cells into antibody-secreting plasma cells [70]. In contrast, intracellular galectin-3 facilitates a memory B-cell phenotype [71], suggesting opposite regulation of B-cell fate by different members of the galectin family.

Lymphoid progenitors committed to become T cells migrate to the thymus, in which they undergo differentiation, selection and proliferation, before exiting and populating the peripheral lymphoid organs as mature naïve T lymphocytes [72]. During their migration in the thymic parenchyma, thymocytes undergo a complex process of differentiation involving developmental stages based on the expression of CD4 and CD8 co-receptors. Interactions between thymocytes and the thymic environment, especially thymic epithelial cells (TECs), are essential for their selection and development into mature T lymphocytes. Among numerous components contributing to the functionality of the thymic environment (e.g. chemokines, extracellular matrix), galectins were proposed to actively participate in thymocyte deletion. Pioneer work demonstrated that galectin-1 is abundantly synthesized by TECs and contributes to TEC-induced thymocyte apoptosis [73], coincident with cellular redistribution of CD45 together with CD3, and of CD43 together with CD7 [74]. More recently, galectin-3 was found to induce T-cell death through binding to distinct cell surface glycoproteins. Although galectin-1 and 3 are both expressed and secreted by TECs, suggesting a potential redundancy in their pro-apoptotic activity in the thymus, the local concentrations of the two galectins, their specificity and affinity for saccharide ligands, as well as their capacity to form high-ordered lattices [75] may contribute to differential receptor recognition, thus allowing greater selectivity in induction of apoptosis of specific thymocyte subsets [15]. On the other hand, it has been shown that galectin-3 is predominantly secreted in the medullary environment, in which it is postulated to disrupt adhesive interactions between thymocytes and TECs [76] and to regulate migration and selection of thymocytes [77]. Like galectin-1 and galectin-3, galectin-8 and galectin-9 have been also shown to trigger death of developing thymocytes [78,79]. However, each individual member of the galectin family can deliver different apoptotic signals by targeting different subsets of developing thymocytes. Whereas galectin-1 kills double-negative ($CD4^-CD8^-$) and double-positive ($CD4^+CD8^+$) thymocytes with equal efficiency, galectin-3 preferentially kills double-negative thymocytes and galectin-8 preferentially binds to double positive cells. Interestingly, CD45 and CD71 appear to be involved in galectin-3-induced T-cell death, although CD43 and CD7 have been found to be critical for galectin-1-induced death [15]. Moreover, galectin-1 can shape the T-cell repertoire by inducing rapid and transient activation of extracellular-signal regulated kinase (ERK) activation during negative selection, but antagonizes ERK activity on thymocytes undergoing positive selection [80].

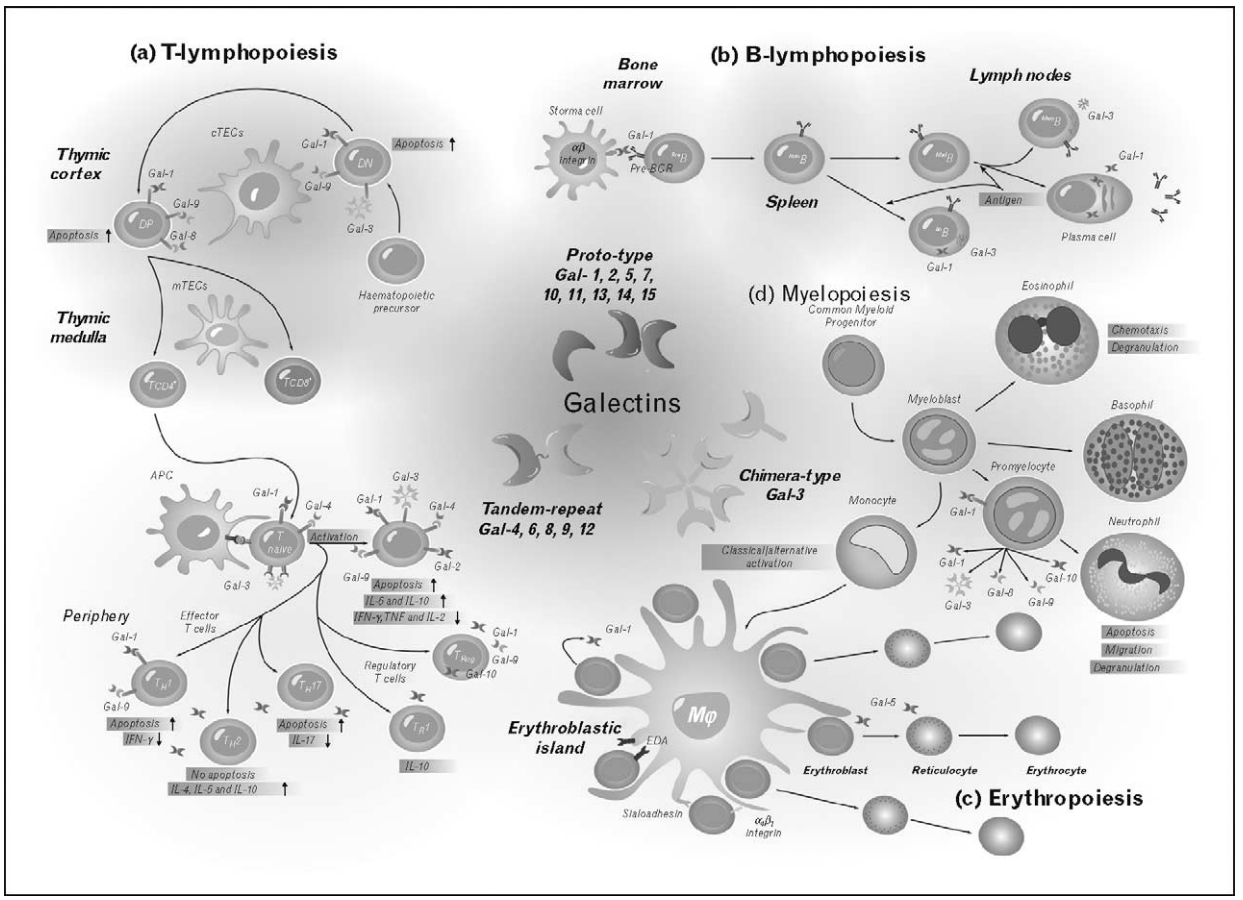
Once in the periphery, T cells undergo dramatic remodeling of cell surface glycans that contribute to T-cell fate through either exposing or masking specific ligands for endogenous galectins [81]. Through cross-linking specific glycoconjugates, galectins modulate T-cell trafficking, activation, differentiation and survival [14,15,20,82]. In addition, galectin-1 and 10 are up-regulated during T-regulatory cell differentiation and substantially contribute to the immunosuppressive activity of these cells [83,84]. Similarly, galectin-1 also contributes to the immunosuppressive activity of mesenchymal stromal stem cells (MSCs) [85,86].

Role of galectins in myelopoiesis and thrombopoiesis

Although galectins play pivotal roles in innate immunity which is a major responsibility of myeloid cells, the function of these lectins during myeloid cell differentiation is less documented (for reviews [27,87]). The human promyelocytic leukemic cell line HL60 was shown to synthesize galectin-1, galectin-3, galectin-8, galectin-9 and galectin-10 whose mRNA expression is differentially up-regulated or down-regulated during differentiation toward eosinophil, monocyte, and neutrophil lineages [88]. Notably, Vas *et al.* [89] showed a biphasic regulation of myeloid cell fate by galectin-1. Whereas low concentrations of this lectin (ng/ml range) increase the formation of granulocyte-macrophage colonies in a lactose-inhibitable fashion, high amounts of galectin-1 (μg/ml range) dramatically reduce the growth of the committed myeloid progenitor cells in a lactose-independent manner [89].

Within peripheral tissues, galectins contribute to regulate the fate of almost all myeloid cells. These multifunctional β-galactoside-binding proteins control dendritic cell maturation, migration and function [19,20,90,91] and influence classical or alternative activation of macrophages [92–94], degranulation of mast cells [95,96] and chemotaxis of eosinophils [97]. In addition, these cells play pleiotropic roles in neutrophil physiology including modulation of activation and signaling, adhesion to extracellular matrix and trafficking [27,98,99].

Figure 2 Control of hematopoietic niches by galectin–glycan lattices



The figure illustrates the way different members of the galectin family contribute to (a) T lymphopoiesis by differentially modulating survival and migration of developing thymocytes, (b) B lymphopoiesis by allowing communication between stromal cells and pre-B cells through bridging glycosylated integrins and the pre-BCR, (c) erythropoiesis by secreting galectin-5 (only found in rat cells of erythroid lineage) and (d) myelopoiesis by differentially secreting different members of the family and positively or negatively influencing growth of myeloid progenitors.

Platelets are derived from larger progenitor cells called megakaryocytes, which are rare, polyploid cells residing within the bone marrow. Platelet production occurs from the tips of long cytoplasmic extensions of megakaryocytes, called proplatelets. It was shown that the quiescent state of megakaryocytes is located in an osteoblastic niche, whereas the release of platelets occurs from megakaryocytes in a vascular niche [100]. The role of the bone marrow microenvironment in the regulation of thrombopoiesis involves differential expression of cellular components and extracellular matrix proteins [101,102] and the secretion of soluble factors [103]. Until now galectins have not been reported to be involved in the regulation of megakaryopoiesis in the bone marrow. However, it was demonstrated that human platelets express galectin-1 and galectin-8 that play key roles in activation, signaling and function of these cells [104]. Galectin-8 could be secreted by endothelial cells but is also released from platelets upon thrombin activation [73].

Conclusion

Figure 2 compiles some of the data reported on the specific interactions between various galectins and different stromal and hematopoietic cells. Since galectins are secreted by both kinds of cells, interact with a large panel of surface glycoproteins (e.g. receptors, integrins) and are involved in various types of processes (e.g. lattice formation, signaling, trafficking), further investigation on the role of galectins in generating appropriate niches during hematopoiesis should be fruitful.

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Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 481).

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