



Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

The immunological potential of galectin-1 and -3

Amy Dhirapong^a, Ana Lleo^a, Patrick Leung^a, M. Eric Gershwin^a, Fu-Tong Liu^{b,*}^a Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis, School of Medicine, 451 Health Sciences Drive, Suite 6510, Davis, CA, 95616, United States^b Department of Dermatology, University of California at Davis, School of Medicine, 3301 C Street, Suite 1400, Sacramento, CA 95816, United States

ARTICLE INFO

Article history:

Received 5 November 2008

Accepted 21 November 2008

Available online xxxx

Keywords:

Apoptosis

Autoimmunity

Galectins

ABSTRACT

A family of β -galactosides-binding proteins, called galectins, have recently emerged as novel molecules with immunoregulatory functions. These proteins are expressed in both inflammatory and non-inflammatory cells including monocytes, macrophages, dendritic cells, mast cells, and B and T cells, giving a broad spectrum of involvement in the immune response. Galectins are uniquely capable of acting both intracellularly and extracellularly, affecting such processes as cell adhesion, signaling, proliferation, differentiation, and apoptosis. Different members of this family have been shown to modulate several pathological processes such as allergic reactions, autoimmunity, and tumor invasion. Therefore, understanding the role of galectins in achieving appropriate proliferative and effector responses to antigens will yield important insights to autoimmune diseases and delineate novel strategies for disease intervention.

© 2008 Published by Elsevier B.V.

Contents

1. Introduction	0
2. Galectins	0
3. Galectins and autoimmunity	0
3.1. Galectin-1	0
3.2. Galectin-3	0
4. Concluding remarks	0
Take-home messages	0
References	0

1. Introduction

The ability to mount an immune response is critically regulated. Despite this regulation, the immune system has the potential to lose tolerance and attack self-tissue, resulting in

autoimmunity. Although clonal deletion and anergy of self-specific lymphocytes are major mechanisms that serve to avoid such a reaction, they do not completely eliminate the possibility of a low-affinity response to self. Due to the prevalence of self-antigen in the body, autoimmune responses can be prolonged and can result in tissue damage that could be life threatening [1–4].

Even though it is well known that autoimmune diseases are a result of a loss of tolerance, the molecular and cellular events that lead to this function remain enigmatic. With these comments in mind, galectins are a family of lectins that bind

* Corresponding author. Department of Dermatology, University of California at Davis, School of Medicine, 3301 C Street, Suite 1400, Sacramento, CA, 95816, United States. Tel.: +1 916 734 6795; fax: +1 916 442 5702.

E-mail address: flu@ucdavis.edu (F.-T. Liu).

to β -galactosides, and regulate the homeostasis and functions of immune repertoire through mechanisms such as apoptosis.

Apoptosis, programmed cell death, is characterized by the retention of the plasma membrane, chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies. Apoptotic cells are engulfed by phagocytes, which recognize multiple components on the surface of the apoptotic cells such as phosphatidylserine (PS) [5]. Ingestion of apoptotic cells leads to a series of events resulting in degradation. The highly efficient elimination of apoptotic cells occurs swiftly and without any tissue damage or inflammation. The effective clearance of these apoptotic bodies is essential for development, homeostasis, and tissue repair. When this system is threatened, autoimmune disorders can arise [6].

Impaired clearance of apoptotic bodies and apoptotic fragments is a potential source of auto-antigens [7]. In this context, the slow or non-existent degradation of phagocytic ingested material may provide neo-antigens, including modified antigens with the potential in genetically susceptible hosts to elicit an autoimmune response. Indeed, apoptotic cells that fail to be effectively engulfed and digested by phagocytes may undergo secondary necrosis [8]. The resultant inflammatory response may include the critical element of innate immunity, which will further amplify inflammation and perhaps facilitate the loss of tolerance. Essentially the process of apoptosis offers a number of venues that have the potential to disrupt tolerance mechanisms. It should be emphasized that any loss of tolerance is dictated not only by the process of apoptosis but also the genetic makeup of the host.

In this review we will discuss the immune properties of galectins, particularly their role in the modulation of apoptosis.

2. Galectins

Galectins are a highly conserved family of proteins that bind to β -galactosides. They are widely distributed and evolutionarily conserved (Fig. 1). Indeed, galectins are characterized by a conserved carbohydrate-recognition domain (CRD), which is folded into five or six B-sheets, and capable of recognizing the structure of N-acetyllactosamine [9]. To date, 15 mammalian galectins have been identified, and classified by the basis of one CRD (galectin-1, -2, -5, -7, -10, -11, -13), two CRDs in tandem (galectin-4, -6, -8, -9, -12), or a chimeric group (galectin-3) [10]. Most galectins have multiple biological effects, including the inflammatory response, cell trafficking, cell survival, cell growth, and chemotaxis. In fact they are expressed on a variety of tissue and cell types [10]. Galectins are located and capable of functioning both extracellularly on the cell surface and medium

and intracellularly in the cytosol and nucleus [11]. Specifically, galectins have been found in primary and secondary lymphoid organs, as well as in circulating immune cells, which further support their role in immunity [12]. Galectins play a vital role in central and peripheral tolerance, and maintaining a functional immune response during apoptosis. This review will focus on the two most ubiquitously expressed and extensively studied members of the galectin family, galectin-1 and -3.

3. Galectins and autoimmunity

3.1. Galectin-1

Galectin-1 is an important contributor in regulating immune function, and maintains T cell homeostasis. It is expressed on both thymic stromal cells and activated T cells [13,14]. In the thymic stromal cell, galectin-1 promotes apoptosis of potential self reactive T cells during development [15]. In the periphery, galectin-1 promotes apoptosis of activated T cells [16], which eliminates the possibility of an exaggerated or inappropriate immune response that can result in unnecessary destruction of self tissue. Thus, the apoptotic function of galectin-1 with activated T cells helps to maintain immune tolerance. In fact, it is clear that without the control of T cell proliferation, altered thymic proliferation can result. For example, in the thymus, a majority of the T cells produced during clonal expansion undergo apoptosis to prevent autoreactive T cells from ever achieving maturity and leaving the thymus. In the periphery, the apoptosis of T cells and other immune cells is important in down-regulating the immune response.

It has been demonstrated that galectin-1 can induce apoptosis specifically through TCR binding and signaling. Upon the engagement of galectin-1 and TCR, apoptosis is induced [17], perhaps through T cell surface reorganization causing cross-linking, and ultimately inducing downstream signal transduction [18]. Thus, galectin-1 is needed for the physical binding and multimerization of TCR to induce apoptosis signals. It has been hypothesized that galectin-1 actually facilitates activated T lymphocytes to apoptosis through the Fas/caspase-8 pathway [19].

Galectin-1 is known to elicit IL-10 production, and contributes to the proliferation of regulatory T cells (Tregs) [20]. It has also been suggested that galectin-1 plays a role in augmenting leukocyte apoptosis, and downregulation of pro-inflammatory cytokines [21,22]. This downregulation curbs a pathogenic Th1 response to a nonpathogenic Th2 response and T regulatory phenotype, with regard to the autoimmune response [23]. Thus, galectin-1 expression can ultimately counteract the harmful Th1 response to a harmless Th2 or T

Human Gal-1	NLCLHFNPRFNAHGD-----ANTIVCNSKD-GGAWG--TEQRE
Human Gal-3	DVAFHFNPRFNENN-----RRVIVCNTKL-DNNWG--REERQ
<i>C. elegans</i> Gal-1A	DVPLHVSVRFD-----EGKIVLNSFS-NGEWG--KEERK
B	DISFHFNPRFD-----EKHVIRNSLA-ANEWG--NEERE
<i>C. elegans</i> Gal-3A	DVPLHLSIRFD-----EGKIVYNAYT-KGTWG--KEERA
B	DIALHFNPRFD-----EKSVVRNSLV-NGEWG--NEERE

Fig. 1. Conserved galectin-1 and 3 CDR regions in human and *C. elegans*. Conserved residues involved in ligand binding are highlighted in red (polar) and blue (hydrophobic). Some galectins have two CDRs in the same polypeptide, designated "A" and "B". The sequences are obtained from Gene Data Bank: Human Galectin-1 (P09382), Human Galectin-3 (P17931), *C. elegans* Galectin-1 (P36573), and *C. elegans* Galectin-3 (Q09581) and from the website, *A genomics resource for animal lectins*.

regulatory response, by inducing IL-10 and downregulation of pro-inflammatory cytokines. Interestingly, galectin-1 is also important in promoting fetomaternal tolerance by altering the balance toward an anti-inflammatory Th2 dominant cytokine profile [24]. This may be due to the higher incidence of galectin-1 binding to Th1 rather than Th2 cells, and therefore negatively regulating Th1 mediated responses by inducing cell death [25].

The pro-apoptotic properties of galectin-1 remain somewhat controversial. It has been demonstrated that the effect of galectin-1 on T cells is not apoptotic but rather, prepares the cells for phagocytic recognition by inducing PS exposure [26,27]. This induction however, is not followed by cell death.

3.2. Galectin-3

Galectin-3 has the unique ability to act in a dual manner. It can protect cells from apoptosis or stimulate cell death depending on whether the protein is functioning intracellularly or extracellularly. Extracellular galectin-3 can induce apoptosis in activated T cells. Treatment of T cells with extracellular recombinant galectin-3 induces apoptosis secondary to caspase pathway activation [28]. For example, treatment of cells with the deletion mutant of extracellular recombinant galectin-3 will not activate the caspase pathway. It has also been shown that galectin-3 directly initiates thymocyte and T cell death, therefore potentially immunosuppressive [29]. In fact T cells treated with recombinant galectin-3 undergo cell death as measured by two cell viability assays that detect increased DNA fragmentation [27]. Similarly, the addition of galectin-3 leads to an increased rate of T cell apoptosis compared to control [30]. Evidently, galectin-3 is pro-apoptotic and therefore important in maintaining tolerance.

Extracellular galectin-3 also plays an important role in the inflammatory response, which also can contribute to autoimmune diseases. The use of galectin-3 knockout mice, has revealed that such mice have an enhanced inflammatory cytokine production in response to LPS [31]. This effect is reduced when galectin-3 is pre-incubated with LPS, before injection into mice, suggesting that the binding of galectin-3 to LPS is the mechanism for this modulation. The addition of galectin-3 can lead to direct mast cell degranulation, resulting in the release of inflammatory cytokines and a prolonged inflammatory response [32,33]. Treatment of mast cells with recombinant galectin-3 also results in apoptosis [34]. These findings define the inflammatory pathways by which galectin-3 can contribute to an exacerbated inflammatory response.

Intracellularly, galectin-3 protects cells from apoptosis and promotes T cell proliferation [35,36]. A number of studies have demonstrated the protective property of galectin-3. For example, cells expressing transfected galectin-3 proliferate faster than control transfectants. In addition, galectin-3 may also suppress apoptosis by interfering with the apoptotic pathway [36]. The overexpression of galectin-3 protects T cells from toxins that induce apoptosis [37]. Mitochondrial homeostasis was maintained, with no signs of blebbing, cell retraction, or rounding, thus protecting cells from death. These data have implication for autoimmunity but rigorous experiments remain to be performed.

In addition to modulating T cell homeostasis, galectin-3 regulates immune cell survival, migration, and cytokine release. In macrophages, galectin-3 also serves a dual role.

Table 1

Immunological effects of galectin-1 and -3

	Immunological effects	Reference
Galectin-1		
<i>in vitro</i>	Induces apoptosis in thymocytes	[15]
	Promotes apoptosis in activated T cells	[16]
	Promotes TCR engagement resulting in enhanced apoptosis	[17]
	Sensitizes T cells to cell death	[19]
	Induces apoptosis in T cells and the induction of IL-10	[20]
	Downregulates pro-inflammatory cytokines	[22]
<i>in vivo</i>	Skewing immune response to a Th2 or T regulatory response	[23]
Galectin-3		
<i>in vitro</i>	Induces apoptosis in activated T cells	[28]
	Induces apoptosis of both thymocytes and T cells	[29]
	Increase apoptosis in T cells	[27,30]
	Induces mast cell apoptosis	[34]
	Protects T cells from apoptosis	[36,37]
	Increases T cell growth	[36]
<i>in vivo</i>	Chemoattractant for macrophages	[39]
	Negatively regulates LPS-mediated inflammation	[31]
	Can contribute to mast cell degranulation	[33]
	Positive regulator of the inflammatory response	[38]
	Essential for effective phagocytosis by macrophages	[40]

One group of researchers [38] found that in macrophages from galectin-3 knockout mice were more sensitive to apoptotic stimuli. Knockout mice also exhibit reduced inflammatory responses measured by decreased numbers of leukocyte infiltrates. Furthermore, the addition of recombinant galectin-3 acts as a chemoattractant for macrophages in a dose dependant manner, and leads them to the site of infection, exacerbating the immune response [39]. Galectin-3 plays an important role on the degranulation and cytokine release of mast cells, as demonstrated by studying galectin-3 knockout mice [33]. Finally, galectin-3 was also found essential for effective phagocytosis by macrophages [40]. Macrophages of galectin-3 knockout mice had a delayed phagocytic response and ingested fewer cells than the wild type mice in the same period. The quick and efficient removal of apoptotic cells is crucial in preventing secondary necrosis, which can lead to autoimmunity (Table 1).

4. Concluding remarks

Maintaining apoptosis is pivotal in the regulation of the immune response. There are clearly multiple overlapping and often promiscuous pathways involved in this regulation. We emphasize the conservation of galectins to highlight their contribution in the clearance of apoptotic cells. However, because galectins are multifunctional and diverse, some questions emerge regarding their true function. These questions stem from the current techniques of studying the extracellular and intracellular role of galectins. Extracellular functions are investigated by adding the galectins exogenously. However, high concentrations are often needed to demonstrate the effects of the galectins. This may simply be due to relatively low efficiency of galectins to deliver an activation signal to the cell. Intracellular functions are studied by extrinsic control of gene expression. The issue that arises with this approach is determining whether the *in vitro* data can be correctly translated to *in vivo* systems. Thus, the use of

galectin knockout mice can provide valuable information on the individual roles of galectins and also contributing to bridging the gap between *in vitro* results and clinical data.

Take-home messages

- Apoptosis plays a crucial role in the pathogenesis of autoimmune diseases, by maintaining T cell homeostasis and the efficient removal of dying cells seems to be essential for the maintenance of tolerance.
- Galectins regulate various cellular processes, including apoptosis and are found in primary and secondary lymphoid organs, as well as circulating immune cells, supporting their role in immunity.
- Galectin-1 has been shown to induce apoptosis in both thymic and activated T cells.
- Galectin-3 has a dual role in both pro- and anti-apoptotic pathways depending on whether it is located intracellularly or extracellularly.

References

- [1] Selmi C. One year in autoimmunity. *Autoimmun Rev* Nov 2007;7(1):85–93.
- [2] Gershwin ME. The mosaic of autoimmunity. *Autoimmun Rev* Jan 2008;7(3):161–3.
- [3] Abbas AK, Lohr J, Knoechel B. Balancing autoaggressive and protective T cell responses. *J Autoimmun* Mar–May 2007;28(2–3):59–61.
- [4] Tsonis IA, Avrameas S, Moutsopoulos HM. Autoimmunity and pathophysiology. *J Autoimmun* Dec 2007;29(4):203–5.
- [5] Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* Apr 1 1992;148(7):2207–16.
- [6] Green DR. Apoptotic pathways: the roads to ruin. *Cell Sep* 18 1998;94(6):695–8.
- [7] Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, et al. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* May 2004 21;304(5674):1147–50.
- [8] Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med* Feb 7 2000;191(3):423–34.
- [9] Rabinovich GA. Galectins: an evolutionarily conserved family of animal lectins with multifunctional properties; a trip from the gene to clinical therapy. *Cell Death Differ* Aug 1999;6(8):711–21.
- [10] Hirabayashi J, Hashidate T, Arata Y, Nishi N, Nakamura T, Hirashima M, et al. Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. *Biochim Biophys Acta Sep* 19 2002;1572(2–3):232–54.
- [11] Hughes RC. The galectin family of mammalian carbohydrate-binding molecules. *Biochem Soc Trans* Nov 1997;25(4):1194–8.
- [12] Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, et al. Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* Jun 2002;23(6):313–20.
- [13] Baum LG, Pang M, Perillo NL, Wu T, Delegeane A, Uittenbogaart CH, et al. Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. *J Exp Med* Mar 1 1995;181(3):877–87.
- [14] Blaser C, Kaufmann M, Muller C, Zimmermann C, Wells V, Mallucci L, et al. Beta-galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. *Eur J Immunol* Aug 1998;28(8):2311–9.
- [15] Perillo NL, Uittenbogaart CH, Nguyen JT, Baum LG. Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J Exp Med* May 19 1997;185(10):1851–8.
- [16] Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. *Nature* Dec 14 1995;378(6558):736–9.
- [17] Vespa GN, Lewis LA, Kozak KR, Moran M, Nguyen JT, Baum LG, et al. Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. *J Immunol* Jan 15 1999;162(2):799–806.
- [18] Reich Z, Boniface JJ, Lyons DS, Borochov N, Wachtel EJ, Davis MM. Ligand-specific oligomerization of T-cell receptor molecules. *Nature* Jun 5 1997;387(6633):617–20.
- [19] Matarrese P, Tinari A, Mormone E, Bianco GA, Toscano MA, Ascione B, et al. Galectin-1 sensitizes resting human T lymphocytes to Fas (CD95)-mediated cell death via mitochondrial hyperpolarization, budding, and fission. *J Biol Chem* Feb 25 2005;280(8):6969–85.
- [20] van der Leij J, van den Berg A, Harms G, Eschbach H, Vos H, Zwiers P, et al. Strongly enhanced IL-10 production using stable galectin-1 homodimers. *Mol Immunol* Jan 2007;44(4):506–13.
- [21] Santucci L, Fiorucci S, Rubinstein N, Mencarelli A, Palazzetti B, Federici B, et al. Galectin-1 suppresses experimental colitis in mice. *Gastroenterology* May 2003;124(5):1381–94.
- [22] Rabinovich GA, Ariel A, Hershkovich R, Hirabayashi J, Kasai KI, Lider O. Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. *Immunology* May 1999;97(1):100–6.
- [23] Toscano MA, Commodaro AG, Ilarregui JM, Bianco GA, Liberman A, Serra HM, et al. Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. *J Immunol* May 15 2006;176(10):6323–32.
- [24] Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, et al. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* Dec 2007;13(12):1450–7.
- [25] Toscano MA, Bianco GA, Ilarregui JM, Croci DO, Correale J, Hernandez JD, et al. Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. *Nat Immunol* Aug 2007;8(8):825–34.
- [26] Dias-Baruffi M, Zhu H, Cho M, Karmakar S, McEver RP, Cummings RD. Dimeric galectin-1 induces surface exposure of phosphatidylserine and phagocytic recognition of leukocytes without inducing apoptosis. *J Biol Chem* Oct 17 2003;278(42):41282–93.
- [27] Stowell SR, Qian Y, Karmakar S, Koyama NS, Dias-Baruffi M, Leffler H, et al. Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. *J Immunol* Mar 1 2008;180(5):3091–102.
- [28] Fukumori T, Takenaka Y, Yoshii T, Kim HR, Hogan V, Inohara H, et al. CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. *Cancer Res* Dec 1 2003;63(23):8302–11.
- [29] Stillman BN, Hsu DK, Pang M, Brewer CF, Johnson P, Liu FT, et al. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J Immunol* Jan 15 2006;176(2):778–89.
- [30] Peng W, Wang HY, Miyahara Y, Peng G, Wang RF. Tumor-associated galectin-3 modulates the function of tumor-reactive T cells. *Cancer Res* Sep 1 2008;68(17):7228–36.
- [31] Li Y, Komai-Koma M, Gilchrist DS, Hsu DK, Liu FT, Springall T, et al. Galectin-3 is a negative regulator of lipopolysaccharide-mediated inflammation. *J Immunol* Aug 15 2008;181(4):2781–9.
- [32] Frigeri LG, Zuberi RI, Liu FT. Epsilon BP, a beta-galactoside-binding animal lectin, recognizes IgE receptor (Fc epsilon RI) and activates mast cells. *Biochemistry* Aug 3 1993;32(30):7644–9.
- [33] Chen HY, Sharma BB, Yu L, Zuberi R, Weng IC, Kawakami Y, et al. Role of galectin-3 in mast cell functions: galectin-3-deficient mast cells exhibit impaired mediator release and defective JNK expression. *J Immunol* Oct 15 2006;177(8):4991–7.
- [34] Suzuki Y, Inoue T, Yoshimaru T, Ra C. Galectin-3 but not galectin-1 induces mast cell death by oxidative stress and mitochondrial permeability transition. *Biochim Biophys Acta* May 2008;1783(5):924–34.
- [35] Lin HM, Pestell RG, Raz A, Kim HR. Galectin-3 enhances cyclin D(1) promoter activity through SP1 and a cAMP-responsive element in human breast epithelial cells. *Oncogene* Nov 14 2002;21(52):8001–10.
- [36] Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc Natl Acad Sci U S A* Jun 25 1996;93(13):6737–42.
- [37] Matarrese P, Tinari N, Semeraro ML, Natoli C, Iacobelli S, Malorni W. Galectin-3 overexpression protects from cell damage and death by influencing mitochondrial homeostasis. *FEBS Lett* May 19 2000;473(3):311–5.
- [38] Hsu DK, Yang RY, Pan Z, Yu L, Salomon DR, Fung-Leung WP, et al. Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. *Am J Pathol* Mar 2000;156(3):1073–83.
- [39] Sano H, Hsu DK, Yu L, Apgar JR, Kuwabara I, Yamanaka T, et al. Human galectin-3 is a novel chemoattractant for monocytes and macrophages. *J Immunol* Aug 15 2000;165(4):2156–64.
- [40] Sano H, Hsu DK, Apgar JR, Yu L, Sharma BB, Kuwabara I, et al. Critical role of galectin-3 in phagocytosis by macrophages. *J Clin Invest* Aug 2003;112(3):389–97.