

MINI REVIEW

Ah, sweet mystery of death! Galectins and control of cell fate

Joseph D. Hernandez and Linda G. Baum¹

Department of Pathology and Laboratory Medicine, Johnson Comprehensive Cancer Center, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90095 USA

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Control of cell death is critical in eukaryotic development, immune system homeostasis, and control of tumorigenesis. The galectin family of lectins is implicated in all of these processes. Other families of molecules function as death receptors or death effectors, but galectins are uniquely capable of acting both extracellularly and intracellularly to control cell death. Extracellularly, galectins cross-link glycan ligands to transduce signals that lead directly to death or that influence other signals regulating cell fate. Intracellular expression of galectins can modulate other signals controlling cell viability. Individual galectins can act on multiple cell types, and multiple galectins can act on the same cell. Understanding how galectins regulate cell viability and function will broaden our knowledge of the roles of galectins in basic biological processes and facilitate development of therapeutic applications for galectins in autoimmunity, transplant-related disease, and cancer.

Key words: apoptosis/galectin/glycosyltransferase/immune system/T cell

Apoptosis: a matter of life and death for multicellular organisms

Programmed cell death, or apoptosis, is indispensable for proper development of multicellular organisms. Cell death shapes the proliferating mass of cells into tissues and shapes tissues into organs (Meier *et al.*, 2000). In the mature organism, cell death plays a critical role in regulating tissue homeostasis. Dysregulation of cell death can cause disease; excess cell death is associated with immunodeficiency and neurodegenerative disorders; and diminished cell death is associated with autoimmunity and cancer (Thompson, 1995).

To maintain the critical balance between cell proliferation and cell death, distinct families of proteins that regulate cell death have evolved. These include death-inducing ligands, death receptors, and intracellular regulators of death pathways. To date, only two families of proteins have been described as

death-inducing ligands: the tumor necrosis factor (TNF) family of proteins and the galectin family (Zimmermann and Green, 2001; Rabinovich *et al.*, 2002b). TNF ligands bind to cognate TNF receptor polypeptides to initiate cell death. In contrast, proapoptotic galectins bind to specific saccharide ligands on cell surface glycoproteins and/or glycolipids to initiate cell death. Inside the cell, additional families of proteins promote or prevent death initiated by extracellular death ligands and receptors. The Bcl family of proteins is the best characterized family of intracellular death regulators, and it contains numerous pro- and anti-death members (Hengartner, 2000). Similar to the Bcl family of proteins, galectins also function intracellularly to promote cell survival or cell death (Yang *et al.*, 1996; Kuwabara *et al.*, 2002). Galectins are unique among molecules regulating cell viability because they act both outside the cell to initiate death signals and inside the cell to regulate susceptibility to death.

Galectins: old dogs learning new tricks?

The galectins are an ancient family of carbohydrate binding proteins found in multicellular organisms from fungi to mammals (Cooper and Barondes, 1999; Muller, 2001). Galectin family members are defined by a conserved carbohydrate recognition domain (CRD) with a canonical amino acid sequence and an affinity for β -galactosides (Barondes *et al.*, 1994). Fourteen galectins have been identified in mammals, and some organisms such as *Caenorhabditis elegans* may have many more (Cooper and Barondes, 1999; Rabinovich *et al.*, 2002a). The evolutionary conservation of galectins likely reflects the roles of galectins in cellular processes essential for the development and function of multicellular organisms, including cell adhesion, migration, differentiation, proliferation, and death (Cooper and Barondes, 1999; Perillo *et al.*, 1998; Leffler, 2001; Goldring *et al.*, 2002).

The carbohydrate binding activity of galectins is essential for many of the family's functions. Galectins can be divided into three groups based on structure: monovalent galectins containing a single CRD that may form homodimers to become functionally bivalent, bivalent tandem repeat galectins possessing two CRDs, and chimeric galectins with a single CRD and unique amino terminus (Rabinovich *et al.*, 2002b). The multivalent nature of galectins facilitates glycan cross-linking believed to be essential in initiating cell signals, including signals leading to death (Bourne *et al.*, 1994; Gupta *et al.*, 1996; Perillo *et al.*, 1995; Leffler, 2001). The basic ligand recognized by the conserved CRD is N-acetylglucosamine (LacNAc), Gal β 1,4GlcNAc, or Gal β 1,3GlcNAc, found on the

¹To whom correspondence should be addressed; E-mail: lbaum@mednet.ucla.edu

termini of Asn N-linked and Ser/Thr O-linked oligosaccharides on numerous glycoproteins. However, various galectin family members bind to modified LacNAc ligands with distinct affinities (Leffler and Barondes, 1986). Differing affinities for more complex saccharides may result in part from structural differences in the CRDs among family members (Rini and Lobsanov, 1999).

The biological significance of specific carbohydrate ligand recognition by various galectin family members is not completely understood but may in part explain the preference of individual galectins for different glycoprotein counterreceptors. For example, both galectin-1 (gal-1) and galectin-3 (gal-3) bind to the lysosomal associated membrane proteins, laminin, and the CD3/T cell receptor (TCR) complex (Pace *et al.*, 1999; Demetriou *et al.*, 2001; Hughes, 2001). However, gal-1 specifically recognizes CD2, CD4, CD7, CD43, and CD45, whereas gal-3 binds to IgE, Fc receptors, CD66, and CD98 (Pace *et al.*, 1999; Walzel *et al.*, 2000; Hughes, 2001). The two CRDs of gal-4 have different preferences for carbohydrate ligands, suggesting that bivalent galectins may cross-link different ligands (Oda *et al.*, 1993). Recognition of unique glycan ligands probably allows different galectins to exert distinct biological effects in various tissues.

Numerous galectins influence cell fate decisions (Table I). Gal-1, -7, -8, -9, and -12 are all proapoptotic (Table II). Some proapoptotic galectins, such as gal-1 and gal-9, directly initiate death by cross-linking cell surface receptors, whereas intracellular expression of other galectins, such as gal-7, potentiates other death signals. Interestingly, gal-3 is the only chimeric galectin identified in mammals, and is also the only antiapoptotic family member (Table I).

Galectins affect cell fate decisions in a variety of tissues and cell types. For example, gal-1 kills T cells, B cells, and prostate and breast cancer cell lines (Table I), suggesting that gal-1 may recognize a common carbohydrate ligand on diverse cell surface receptors to initiate a common intracellular death pathway. Similarly, gal-3 protects both T cells and breast cancer cells from apoptosis (Table I). Multiple galectins have also been shown to influence viability in a single cell type. For example, gal-1 and gal-9 are both proapoptotic for T cells, and gal-3 protects T cells from various apoptotic stimuli (Table I). Complex expression patterns of galectins and their carbohydrate ligands may allow temporal regulation of cell viability during development, tissue remodeling, and inflammation.

A necessary end: galectins trigger T cell death

Cell death is critical for proper T cell development in the thymus. Over 90% of developing T cells (thymocytes) die in the thymus while learning to distinguish self from nonself. Thymocytes die because the cells either fail to rearrange a functional antigen receptor (failure of positive selection/nons-election) or rearrange an antigen receptor that is self-reactive (negative selection) (Kishimoto and Sprent, 2000). Therefore cell death prevents the production of nonfunctional and autoreactive T cells. These selection events occur primarily among immature CD4/CD8 double-positive thymocytes in the cortex (Kishimoto and Sprent, 2000). Both gal-1 and gal-9 are expressed by thymic epithelial cells in the cortex that mediate thymic selection events, and both gal-1 and gal-9 kill thymocytes (Baum *et al.*, 1995; Perillo *et al.*, 1997; Wada *et al.*, 1997). Double-positive cortical thymocytes are the thymocyte subset most susceptible to gal-1 induced death (Figure 1) (Perillo *et al.*, 1997; Vespa *et al.*, 1999). Gal-1 and gal-9 may therefore participate in selection events critical for the development of a functional and self-tolerant T cell repertoire.

Cell death also regulates the T cell response to antigen in peripheral tissues. Following an infection or other immune challenge, cell death eliminates 90% of antigen-specific T cells (Sprent and Tough, 2001). Multiple pathways contribute to the effective clearance of activated T cells following an immune response. Gal-1 and gal-9 are widely expressed by cells in tissues that T cells invade during the immune response, such as endothelia, fibroblasts, lung epithelial cells, and myocytes (Wada and Kanwar, 1997; Perillo *et al.*, 1998). Gal-1 is also expressed by antigen presenting cells such as macrophages and B cells (Rabinovich *et al.*, 1998; Zuniga *et al.*, 2001). Both gal-1 and gal-9 may contribute to the elimination of antigen-activated peripheral T cells because gal-1 killed activated but not resting human T cells and gal-9 killed activated T cells in a murine nephritis model (Perillo *et al.*, 1995; Tsuchiyama *et al.*, 2000). In addition, gal-1 is also expressed by activated T cells, suggesting a potential autocrine suicide mechanism to eliminate activated T cells at the end of the immune response (Blaser *et al.*, 1998). Galectins may therefore regulate immune homeostasis both during development in the thymus and in the periphery.

Living in a dangerous world: regulating galectin-induced cell death

How do cells survive when they are constantly surrounded by galectins? Susceptibility to galectins is controlled by the cell

Table I. Cell fate regulation by galectins in a variety of tissues

	Apoptosis	Cell cycle effects	References
Galectin-1	T cells, B cells, prostate and breast cancer cell lines	S/G2 arrest: T cells, breast cancer, neuroblastoma Proliferative: endothelia, fibroblasts	Sanford and Harris-Hooker, 1990; Wells and Mallucci, 1991; Perillo <i>et al.</i> , 1995; Adams <i>et al.</i> , 1996; Blaser <i>et al.</i> , 1998; Novelli <i>et al.</i> , 1999; Fouillit <i>et al.</i> , 2000
Galectin-7	keratinocytes, carcinoma	unknown	Bernerd <i>et al.</i> , 1999; Kuwabara <i>et al.</i> , 2002
Galectin-8	carcinoma	unknown	Hadari <i>et al.</i> , 2000
Galectin-9	thymocytes	unknown	Wada and Kanwar, 1997
Galectin-12	adipocytes, carcinoma	G1 arrest: carcinoma	Yang <i>et al.</i> , 2001; Hotta <i>et al.</i> , 2001
Galectin-3	anti-apoptotic: T cells, breast cancer	Proliferative: T cells	Yang <i>et al.</i> , 1996; Akahani <i>et al.</i> , 1997; Joo <i>et al.</i> , 2001

Table II. Autoimmune disease models treated with galectins

Animal model for	Galectin used	Amelioration		Antigen-specific proliferation	T cell apoptosis	Cytokine profile
		Clinical	Histologic			
Myasthenia gravis ^a	gal-1	Yes	ND	decreased	ND	ND
Multiple sclerosis ^b	gal-1	Yes	Yes	decreased	ND	ND
Rheumatoid arthritis ^c	gal-1	Yes	Yes	ND	increased	T _H 1 → T _H 2
Hepatitis ^d	gal-1	Yes	Yes	ND	increased	T _H 1 → T _H 2
Glomerulonephritis ^e	gal-1, 3, 9	Yes	Yes	ND	increased	ND
Graft vs. host disease ^f	gal-1	Yes	Yes	decreased	ND	T _H 1 → T _H 2

^aExperimental autoimmune myasthenia gravis (Levi *et al.*, 1983).

^bExperimental autoimmune encephalitis (Offner *et al.*, 1990).

^cCollagen-induced arthritis (Rabinovich *et al.*, 1999b).

^dCon A-induced hepatitis (Santucci *et al.*, 2000).

^eNephrotoxic serum nephritis (Tsuchiyama *et al.*, 2000).

^fAllogeneic bone marrow transplantation (Delioukina *et al.*, 1999).

and may be regulated at three levels: (1) synthesis and modification of glycan ligands by glycosyltransferases, (2) presentation of glycan ligands by specific glycoprotein counterreceptors, and (3) intracellular signaling pathways initiated by galectin binding to glycoprotein counterreceptors.

Synthesis and modification of glycan ligands by glycosyltransferases

Gal-1 binds to the basic ligand LacNAc, as discussed, but binds with greater avidity to glycan ligands containing multiple LacNAc units (Leffler and Barondes, 1986; Merkle and Cummings, 1988; Solomon *et al.*, 1991). Multiple LacNAcs may be presented on the branches of N-glycans or occur as polyN-acetyllactosamine (polyLacNAc) chains on either N- or O-linked glycans (Figure 2). Generation of poly-LacNAc sequences is regulated in part by the family of core 2 β -1,6-N-acetylglucosaminyltransferase (C2GnT) branching enzymes for O-glycans and β -1,6-N-acetylglucosaminyltransferase V (GNTV) branching enzyme for N-glycans (Figure 2A,B) (Cummings and Kornfeld, 1984; Yousefi *et al.*, 1991; Bierhuizen *et al.*, 1994). Regulated expression of glycosyltransferases during development and activation, creating polyLacNAc ligands, may therefore determine cell susceptibility to gal-1 *in vivo*.

In the immune system, the C2GnT enzyme and core 2 O-glycans are expressed by gal-1-susceptible T cell populations (Figure 1A,B). Cortical thymocytes undergoing selection express C2GnT and bear core 2 O-glycans on cell surface glycoproteins, whereas mature thymocytes do not (Baum *et al.*, 1995). Likewise, antigen-stimulated T cells bear core 2 O-glycans, whereas naive and memory T cells do not (Piller *et al.*, 1988; Harrington *et al.*, 2000; Priatel *et al.*, 2000). Expression of C2GnT type I (C2GnT-I) in a C2GnT-negative, gal-1-resistant T cell line rendered the cell line susceptible to gal-1-induced death, and thymocytes from transgenic mice overexpressing C2GnT-I demonstrated increased sensitivity to gal-1-induced death (Galvan *et al.*, 2000b). Although C2GnT-I transgenic mice demonstrate altered T cell responses, C2GnT-I^{-/-} mice have been generated and have no documented T cell defects (Tsuboi and Fukuda, 1997; Ellies *et al.*, 1998). However, multiple isoforms of the C2GnT have been identified,

including one that appears to be T cell-specific, suggesting that multiple C2GnT knockouts will be necessary to understand the interaction of gal-1 and core 2 O-glycans in T cell development and homeostasis (Schwientek *et al.*, 2000).

It remains to be determined whether GNTV has a similar role in regulating gal-1 death. In contrast to C2GnT, GNTV and its corresponding saccharide structure are expressed throughout thymic development (data not shown) (Figure 1A). GNTV is expressed by resting peripheral T cells, but the expression and activity of GNTV are up-regulated with activation (Figure 1B) and may modify specific glycoprotein acceptor substrates (Lemaire *et al.*, 1994; Demetriou *et al.*, 2001). GNTV has been shown to regulate antigen recognition by T cells, at least in part through the creation of cell surface ligands for gal-3 (Demetriou *et al.*, 2001). GNTV may also create ligands for gal-1, as indirectly inhibiting GNTV activity by swainsonine treatment of activated human peripheral T cells rendered the cells partially resistant to gal-1-induced death (Perillo *et al.*, 1995). However, GNTV is not essential for death, because a GNTV negative murine T cell line, PHA^R 2.9, was susceptible to gal-1 death (Galvan *et al.*, 2000b). These results may reflect other differences between primary and transformed cells, leaving unresolved the role of GNTV in gal-1-induced T cell death.

T cell susceptibility to gal-1 may be additionally regulated by glycosyltransferases competing for acceptor substrates to limit carbohydrate ligand synthesis. ST3Gal I competes with C2GnT for core 1 O-glycan substrates and thus inhibits the addition of O-linked polyLacNAc ligands for gal-1 (Figure 2B) (Priatel *et al.*, 2000; Dalziel *et al.*, 2001). Interestingly, a provocative inverse correlation exists between ST3Gal I expression and gal-1 susceptibility. ST3Gal I is not expressed by gal-1-susceptible immature cortical thymocytes but is expressed by gal-1-resistant mature medullary thymocytes (Figure 1A) (Gillespie *et al.*, 1993). Expression of other enzymes potentially competing for core 1 O-glycan substrates, such as the ST6GalNAc IV, have also been documented to increase during early T cell activation and may therefore protect T cells from gal-1 early during the immune response (Kaufmann *et al.*, 1999).

Glycosyltransferases may also modify LacNAc ligands to block gal-1 binding and reduce T cell susceptibility to gal-1.

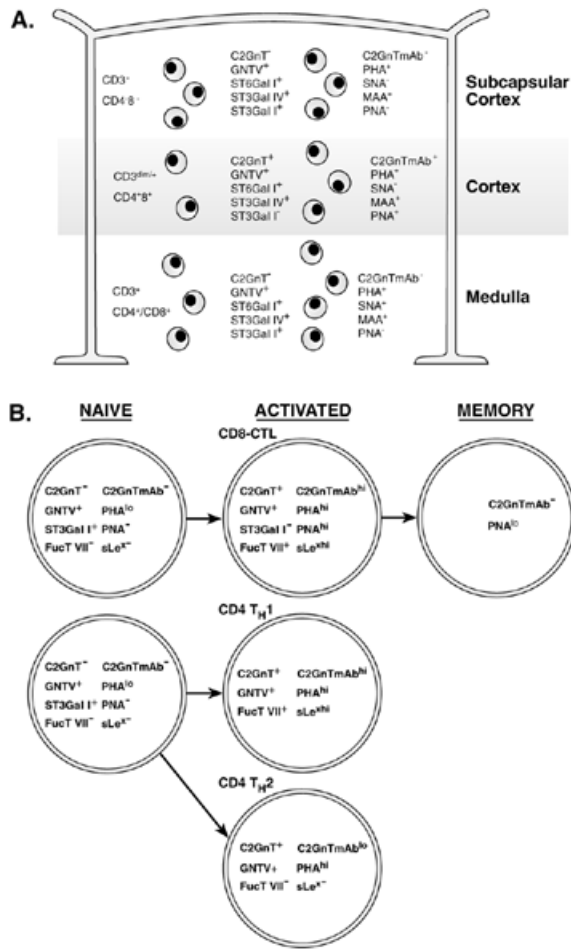


Fig. 1. Glycosyltransferase expression during T cell development and activation. (A) T cell development in the human thymus. T cell precursors enter from the bone marrow and migrate from the subcapsular cortex (least mature) to the medulla (most mature) while undergoing T cell receptor rearrangement and selection. Expression of developmental markers is shown at left. Expression of glycosyltransferase mRNA at the various stages is shown in the middle column (+ or -). The presence of corresponding carbohydrate epitopes, as detected by lectin and monoclonal antibody staining, is depicted in the right column (+ or -). RNA expression may not necessarily reflect lectin binding profiles because of possible posttranscriptional regulation, expression of specific acceptor substrates, or competition with other enzymes. (B) T cell activation and differentiation in the mouse. In each cell the left column shows glycosyltransferase mRNA expression. The right column shows the presence of corresponding carbohydrate epitopes, as detected by lectin and monoclonal antibody staining (-, lo, or hi). Specificity of each lectin is given in references: PHA, phytohemagglutinin (Cummings *et al.*, 1982); SNA, *Sambucus nigra* agglutinin (Shibuya *et al.*, 1987); MAA, *Maackia amurensis* agglutinin (Wang and Cummings, 1988); PNA, peanut agglutinin (Sharon, 1983); C2GnTmAb, different monoclonal antibodies that recognize core 2 O-glycans on CD43.

Although some modifications of LacNAc are permissive for gal-1 binding, addition of either sialic acid in an α 2,6 linkage to galactose or of fucose to glucosamine, modifying the LacNAc ligand, can inhibit gal-1 binding (Figure 2C) (Leffler and Barondes, 1986; Pace *et al.*, 1999). SA α 2,6Gal sequences are expressed on mature medullary thymocytes resistant to gal-1 death but not on gal-1 susceptible immature cortical thymocytes (Figure 1A) (Baum *et al.*, 1996). In addition, overexpression of ST6Gal I in a gal-1-susceptible T cell line rendered it resistant to gal-1-induced death (Galvan *et al.*, 2000a). FucT VII

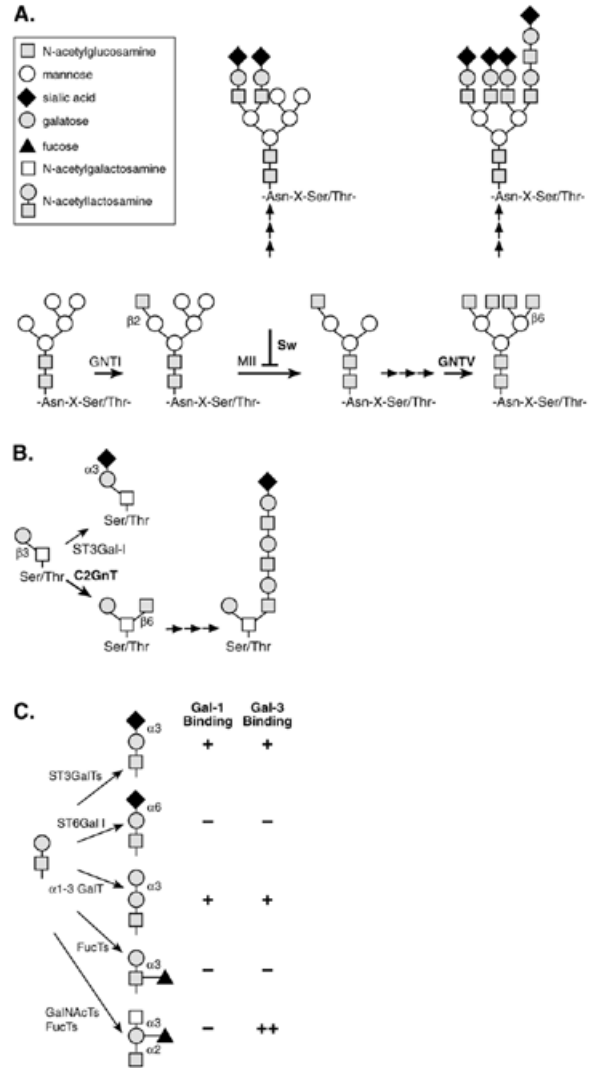


Fig. 2. Synthesis of saccharide ligands for gal-1. PolyLacNAc is a preferred ligand for gal-1. Branched N-glycans may also present multiple LacNAc units that can bind gal-1. Galactose and N-acetylglucosamine units composing a LacNAc disaccharide on N- and O-glycans are shown by stippling. (A) Pathway for N-glycan synthesis. Gal-1 can bind LacNAc units on branched glycans and polyLacNAc chains present on N-glycans. The hybrid glycan structure resulting from swainsonine (Sw) inhibition of mannosidase II activity is shown. The GNTV branching enzyme is shown in bold. (B) Pathway for O-glycan synthesis. The C2GnT branching enzyme, in bold, can control the addition of LacNAc to O-glycans. (C) Modifications of LacNAc. Linkages formed by glycosyltransferases and their effects on gal-1 and gal-3 binding are shown. The figure is not intended to depict precise acceptor substrate specificities for each of the enzymes.

activity is up-regulated on T cell activation (Figure 1B), suggesting that regulated fucosyltransferase activity may similarly control galectin binding and susceptibility to death (Lim *et al.*, 2001). Changes in neuraminidase activity also accompany T cell activation and may unmask glycan ligands by removing sialic acid (Galvan *et al.*, 1998). Thus, the making and masking of cell surface glycan ligands may control galectin binding and triggering of T cell death.

Little is known about how glycosylation may influence other gal-1 functions or the functions of other galectin family members. Individual galectin family members have different

affinities for glycan ligands. For instance, gal-3 demonstrates enhanced binding to Fuc α 1,2[GalNAc α 1,3]Gal β 1,4GlcNAc compared to unmodified LacNAc (Figure 2C) (Leffler and Barondes, 1986; Sato and Hughes, 1992). This suggests that the glycosyltransferases synthesizing these structures may regulate functions of gal-3. A systematic evaluation of the carbohydrate binding specificities of individual galectin family members and an assessment of changes in glycosyltransferase expression during the development and activation of target cells will greatly facilitate our understanding of galectin biology. These are among the goals of the Consortium for Functional Glycomics (<http://glycomics.scripps.edu>).

Presentation of glycan ligands by specific glycoprotein counterreceptors

As already mentioned, different galectins can discriminate among LacNAc-bearing glycoproteins on the cell surface and selectively recognize unique complements of receptor glycoproteins. For example, gal-1 binds a specific subset of T cell glycoproteins: CD2, CD3, CD4, CD7, CD43, and CD45 (Walzel *et al.*, 2000; Pace *et al.*, 1999). In addition, antibodies to GM1 inhibited gal-1 binding to neuroblastoma cells, suggesting that gal-1 may also bind to GM1 or to glycoprotein receptors in close proximity to GM1 (Kopitz *et al.*, 1998). Different cell surface counterreceptors for gal-1 may have distinct functions in signaling the various processes mediated by gal-1.

CD7 is essential for signaling gal-1-mediated T cell death (Pace *et al.*, 2000). Confocal microscopy has demonstrated the colocalization of CD7 with CD43, another gal-1 counterreceptor (Pace *et al.*, 1999). The colocalization of CD43 with CD7 suggests that these two glycoproteins may act in concert to initiate death.

Other gal-1 counterreceptors are not essential for gal-1 initiation of cell death but appear to be regulators of death. Gal-1 binds CD45, a heavily N- and O-glycosylated receptor tyrosine phosphatase (Walzel *et al.*, 1999; Pace *et al.*, 1999; Fouillit *et al.*, 2000; Symons *et al.*, 2000). Early studies using two CD45 negative cell lines implied an essential role for CD45 in signaling gal-1 death (Perillo *et al.*, 1995; Walzel *et al.*, 1999). However, neither study demonstrated restored susceptibility to gal-1 after CD45 reconstitution. In contrast, a recent study found that CD45 regulation of gal-1-induced death depends on the glycosylation state of CD45 (Nguyen *et al.*, 2001). These studies showed CD45 can inhibit gal-1 death but is permissive for death when modified by core 2 O-glycans during T cell development and activation (see previous discussion).

Gal-1-induced T cell death does not require CD2, CD3, or CD4 (Pace *et al.*, 2000). However these counterreceptors may be important for mediating other biological effects of gal-1 on T cells.

Intracellular signaling pathways initiated by gal-1 binding to glycoprotein counterreceptors

Gal-1 binding to T cells results in rapid redistribution of glycoprotein counterreceptors on the T cell surface (Figure 3) (Pace *et al.*, 1999). CD7 and CD43 colocalize in clusters after gal-1 binding that are physically separate from CD3 and CD45 clusters localized to apoptotic membrane blebs (Pace *et al.*, 1999). Counterreceptor clustering appears to be regulated by the presence of specific glycan ligands, such as core 2 O-glycans (Nguyen *et al.*, 2001). These observations suggest that dimeric

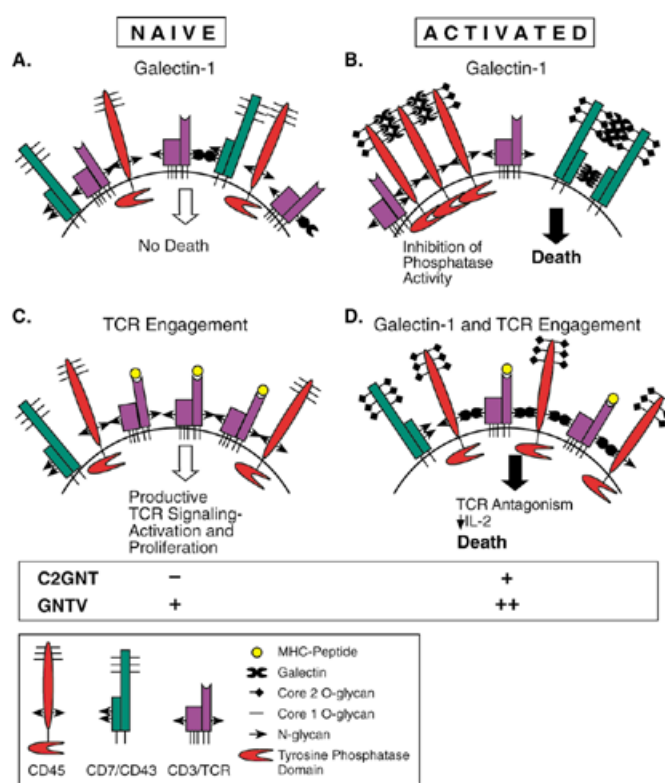


Fig. 3. A model of gal-1 signaling events in peripheral T cells. (A) On naive T cells, gal-1 binding does not result in the clustering of CD3, CD7, CD43, or CD45 or in cell death because the appropriate complement of glycosyltransferases is not expressed. (B) On activated T cells expressing the required glycosyltransferases, gal-1 binding results in the segregation of gal-1 counterreceptors CD45, CD3, CD7, and CD43 into discrete membrane microdomains to initiate a death cascade. Gal-1 clustering of CD45 on activated T cells also results in inhibition of tyrosine phosphatase activity. (C) On naive T cells, TCR engagement results in TCR clustering, proliferation and activation. (D) On activated T cells, concurrent TCR engagement and gal-1 binding decreases TCR clustering, resulting in diminished TCR signaling and death.

gal-1 cross-links multivalent counterreceptors to build a platform initiating the intracellular signals for death (Sacchetti *et al.*, 2001).

What are the intracellular signaling events triggered by gal-1 binding (Figure 3)? CD7 and CD43 associate with tyrosine, serine/threonine, and lipid kinases (Ostberg *et al.*, 1998; Sempowski *et al.*, 1999; Wang *et al.*, 2000). Gal-1 binding induced changes in tyrosine phosphorylation patterns (Vespa *et al.*, 1999; Chung *et al.*, 2000) and increased intracellular calcium as a downstream effect of lipid kinase activation (Walzel *et al.*, 2000). Although increased intracellular calcium does not appear to be necessary for initiation of death, the roles of other signaling pathways initiated by CD7 and CD43 are currently being examined (Pace *et al.*, 2000).

As mentioned, gal-1 binds to core 2 O-glycans and clusters CD45 (Nguyen *et al.*, 2001). CD45 has two cytoplasmic tyrosine phosphatase domains, and clustering of CD45 has been proposed to block access of these domains to phosphorylated substrates (Majeti *et al.*, 1998). Gal-1 binding to lymphocytes reduced CD45 tyrosine phosphatase activity (Walzel *et al.*, 1999; Fouillit *et al.*, 2000). In addition, a pharmacologic inhibitor of

tyrosine phosphatase activity enhanced gal-1-induced death and rendered a CD45⁺ C2GnT⁻ cell line susceptible to death (Nguyen *et al.*, 2001). These observations suggest that CD45 tyrosine phosphatase activity can inhibit gal-1-induced death; however, when C2GnT is expressed during T cell development or following T cell activation, gal-1 can bind to core 2 O-glycans and cluster CD45, inhibiting tyrosine phosphatase activity and allowing the initiation of cell death (Figure 3B).

Additional signaling pathways can be initiated following galectin binding to different cell types. Both gal-1 and gal-3 trigger a calcium flux in T cells (Dong and Hughes, 1996; Pace *et al.*, 2000; Walzel *et al.*, 2000). Gal-1 binding to neutrophils results in increased production of reactive oxygen species (Timoshenko *et al.*, 1997; Almkvist *et al.*, 2002). Gal-1 binding to T cells resulted in increased AP-1 DNA binding activity (Rabinovich *et al.*, 2000). The rapid time course of gal-1-induced death makes it unlikely that *de novo* transcription is required for the initiation of death, but transcription may be required for other gal-1 effects (Pace *et al.*, 1999).

Team players: galectins influence other cell death pathways

Many galectins both potentiate and antagonize cell death pathways in a variety of cell types. Some galectins can act extracellularly to induce apoptosis in concert with other stimuli. For example, addition of exogenous gal-8 induced apoptosis of serum-starved carcinoma cells in a carbohydrate-dependent manner (Hadari *et al.*, 2000). However, most experiments have examined the effects of expressing cDNA encoding various galectins on cellular susceptibility to apoptosis. In this case, the galectins may act intracellularly or extracellularly, depending on whether they are secreted. From these studies, considerable evidence now supports intracellular roles for several galectin family members in regulating cell death. Gal-7 expression is induced by the tumor suppressor p53 and is associated with UVB-induced death of keratinocytes (Bernerd *et al.*, 1999; Polyak *et al.*, 1997). HeLa cells transfected with gal-7 demonstrated enhanced cytochrome c release, enhanced caspase activity, and up-regulated JNK activity in response to various apoptotic stimuli (Kuwabara *et al.*, 2002). In adipocytes, gal-12 expression induced apoptosis, and gal-12 expression was increased during drug-induced adipocyte apoptosis (Hotta *et al.*, 2001).

As mentioned previously, only gal-3 has been found to protect cells from apoptosis. Gal-3 expression in Jurkat T cells inhibited apoptosis induced by Fas, staurosporine, and chemotherapeutic agents (Yang *et al.*, 1996). Expression of gal-3 protected breast cancer cells from death induced by chemotherapeutic agents, nitric oxide, and detachment (Akahani *et al.*, 1997; Kim *et al.*, 1999; Moon *et al.*, 2001). Gal-3 possesses an NWGR motif characteristic of Bcl family members that is necessary for both gal-3 and Bcl-2 antiapoptotic activity (Yang *et al.*, 1996; Akahani *et al.*, 1997). The motif is believed to facilitate the interaction of Bcl family members and, because gal-3 coimmunoprecipitated with Bcl-2 from Jurkat T cells, may facilitate the association of gal-3 with Bcl proteins (Yang *et al.*, 1996). Phosphorylation of gal-3 at ser-6 is required for antiapoptotic activity, and dephosphorylation at ser-6 is required for carbohydrate ligand binding (Mazurek *et al.*,

2000; Yoshii *et al.*, 2002). Similar to Bcl family members, intracellular gal-3 preserved mitochondrial integrity and prevented cytochrome c release from mitochondria in breast cancer cells after challenge with staurosporine and chemotherapeutic agents (Matarrese *et al.*, 2000; Yu *et al.*, 2002). Therefore, although gal-3 can be secreted, the antiapoptotic effect appears to be mediated by intracellular gal-3 (Sato *et al.*, 1993). Exogenous gal-3, however, induced differentiation of a kidney epithelial cell line and proliferation of fibroblasts, demonstrating that extracellular gal-3 can also influence cell fate (Inohara *et al.*, 1998; Hikita *et al.*, 2000).

Secreted gal-3 has also been proposed to regulate TCR signaling by binding glycan ligands synthesized by GNTV (Demetriou *et al.*, 2001). GNTV^{-/-} T cells demonstrated increased TCR clustering on stimulation and T cell hyperactivation compared to wild-type cells (Demetriou *et al.*, 2001). GNTV^{-/-} mice developed autoimmune kidney disease and exhibited increased susceptibility to experimental autoimmune encephalitis, a model for multiple sclerosis (Demetriou *et al.*, 2001). These results imply that gal-3 interacts with the TCR to raise the threshold for T cell activation by limiting TCR clustering. Recent studies have also demonstrated that gal-3 modulates interactions of developing thymocytes with thymic epithelia, suggesting that gal-3 may influence TCR signaling events during thymocyte selection (Villa-Verde *et al.*, 2002).

Gal-1 also influences T cell death resulting from TCR ligation (Figure 3). TCR stimulation and gal-1 treatment acted synergistically to kill T cell lines and thymocytes while inhibiting TCR-induced proliferation (Perillo *et al.*, 1997; Vespa *et al.*, 1999; Chung *et al.*, 2000). Concomitant TCR engagement and gal-1 treatment also killed C2GnT lo CD4/CD8 single-positive thymocytes, a population normally resistant to death induced by gal-1 alone (Vespa *et al.*, 1999). This suggests that death induced by gal-1 alone and death induced by gal-1 and TCR stimulation may proceed via different mechanisms. Miceli and co-workers (Chung *et al.*, 2000) have proposed that gal-1 limits the formation of the immune synapse, a clustering of receptors and signaling molecules in membrane microdomains that is required to transduce TCR signals leading to activation and proliferation (Figure 3C).

Galectin knockouts: beginning to define functions

To investigate the roles of galectins in development and immune regulation, gal-1- and gal-3-null mice have been generated (Poirier and Robertson, 1993; Colnot *et al.*, 1998a). In gal-1^{-/-} mice, olfactory neurons showed altered neurite outgrowth and targeting, demonstrating a role for gal-1 in neural development (Puche *et al.*, 1996). No dramatic effects on immune development were observed in gal-1^{-/-} mice that were otherwise wild type. However, defects in immune homeostasis were observed in gal-3^{-/-} mice. Altered inflammatory cell dynamics during acute peritonitis indicate a role for gal-3 in leukocyte recruitment and/or maintenance (Colnot *et al.*, 1998b; Hsu *et al.*, 2000).

Results obtained from *in vitro* studies, such as the ability of both gal-1 and gal-9 to induce T cell apoptosis, suggest that there may be functional redundancy among galectin family members, although there is not yet clear evidence for redundancy *in vivo*. To examine functional redundancy *in vivo* and

to understand clearly the roles of galectins in various biological processes, mice with mutations in multiple galectins will need to be generated. In addition, detailed analysis of specific biological questions using galectin-null mice crossed onto various genetic backgrounds may also be informative.

Galectins as immunomodulatory agents and therapeutics

What causes autoimmune disease? Self-reactive T cells that trigger autoimmune disease can arise through two mechanisms: a failure to eliminate self reactive T cells during development in the thymus or a breakdown in peripheral T cell tolerance for self-antigens (Kishimoto and Sprent, 2000). Two classes of T cells are associated with the initiation and maintenance of autoimmune disease. T_H1 CD4⁺ cells mediate a cellular inflammatory and cytotoxic T cell response in such diseases as multiple sclerosis and Type I diabetes. T_H2 CD4⁺ cells promote the production of autoantibodies in such diseases as systemic lupus erythematosus. Manipulating the T_H1/T_H2 balance can be therapeutic in many models of autoimmune disease.

Galectins have been used successfully as therapeutics in several T_H1-mediated autoimmune disease models (Table II). Before the effects of gal-1 on T cells were known, gal-1 administration was used to treat animal models of myasthenia gravis and multiple sclerosis. Gal-1 treatment resulted in decreased antigen-induced T cell proliferation in both models, as well as the inability to isolate antigen-specific T cell clones from the multiple sclerosis model, suggesting that deletion or anergy of autoreactive T cells occurred in gal-1-treated animals (Levi *et al.*, 1983; Offner *et al.*, 1990). In some animal models, gal-1 treatment resulted in a decrease in T_H1 cytokines and an increase in T_H2 cytokines (Table II), suggesting that gal-1 may preferentially suppress or delete T_H1 CD4⁺ T cells. How does gal-1 mediate this shift in the immune response? It is unclear, but several possibilities exist. Gal-1 alters T cell receptor signaling and suppresses T_H1 cytokine production, both of which can influence the further differentiation of naive CD4⁺ T cells into T_H1 or T_H2 effector cells (Leitenberg and Bottomly, 1999; Vespa *et al.*, 1999; Rabinovich *et al.*, 1999a; Chung *et al.*, 2000). Alternatively, gal-1 may preferentially bind to and kill T_H1 cells as T_H1 and T_H2 cells display different glycosylation profiles (Figure 1B). Indeed, amelioration of disease in several models correlated with increased T cell apoptosis (Table II), although effects on specific helper T cell subsets were not addressed.

In a model of nephritis, treatment with gal-1, gal-3, or gal-9 effectively ameliorated disease (Tsuchiyama *et al.*, 2000). Do all three galectins function via the same mechanism? Gal-3 and gal-9 may both influence T cell signaling and survival as discussed, but may also affect other immune cells. Gal-3^{-/-} mice displayed defective neutrophil recruitment and decreased macrophage survival; this may influence antigen presentation and the development of an immune response (Colnot *et al.*, 1998b; Hsu *et al.*, 2000). In addition, gal-3 down-regulated IL-5, a T_H2 cytokine, in a variety of cell types (Cortegano *et al.*, 1998). Interestingly, this suggests that gal-1 and gal-3 may have opposing effects on T helper cell cytokine production, just as they have opposing effects on cell death. In addition to directly killing T cells, gal-9 is a chemoattractant and activator of eosinophils, cells important to allergic inflammation

(Matsumoto *et al.*, 2002). Therefore, individual galectins have a variety of effects on multiple cell types, each of which may affect the development of the immune response.

There is still much to be learned about the galectins—the cells they affect, subtleties in glycan ligand preference, the identity of specific counterreceptors, and signaling pathways triggered by the counterreceptors. With this knowledge, various galectins may be useful immunotherapeutics for autoimmune and transplant-related disease or as specific adjuvants to bolster a specific immune response during infection, vaccination, or cancer therapy.

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Abbreviations

C2GnT, core 2 β -1,6-N-acetylglucosaminyltransferase; CRD, carbohydrate recognition domain; GNTV, β -1,6-N-acetylglucosaminyltransferase V; polyLacNAc, polyN-acetyllactosamine; TCR, T cell receptor; TNF, tumor necrosis factor.

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