

# "Galectin-1 Induces Central and Peripheral Cell Death: Implications in T-Cell Physiopathology"

C.E. SOTOMAYOR\* and G.A. RABINOVICH†

*Inmunología. Dpto. Bioquímica Clínica Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Córdoba. Argentina*

The immune system has a remarkable capacity to maintain a state of equilibrium even as it responds to a diverse array of foreign proteins and despite its contact exposure to self-antigens. Apoptosis is one of the mechanisms aimed at preserving the homeostasis after the completion of an immune response, thus returning the immune system to a basal state and warranting the elimination of autoaggressive cells in both central and peripheral lymphoid organs. Targeted deletions in critical genes involved in the apoptotic death machinery together with natural spontaneous mutations have clearly shown the importance of apoptosis in the regulation of the immune response. This complex scenario of stimulatory and inhibitory genes has been enriched with the finding that galectin-1, a 14.5 kDa  $\beta$ -galactoside-binding protein, is able to induce apoptosis of immature cortical thymocytes and mature T cells by cross-linking cell surface glycoconjugates. Galectin-1 is present not only in central and peripheral lymphoid organs, but also at sites of immune privilege. In the present article we will discuss the implications of galectin-1-induced apoptosis in T-cell physiopathology in an attempt to validate its therapeutic potential in autoimmune and inflammatory diseases.

*Keywords:* galectin-1, apoptosis, immunomodulation, macrophages, autoimmunity

## INTRODUCTION

Death, along with growth and differentiation, is a critical part of the life cycle of the cell. Homeostasis control of cell number is thought to be the result of the dynamic balance between cell proliferation and cell death. It is only in the past ten years, that the attention has been focused on the physiological occurrence of cell death and its role in the homeostasis.

Apoptosis or programmed cell death, is a phenomenon that plays a crucial role in a myriad of physiological and pathological processes. This review will

briefly cover some relevant aspects of programmed cell death in the immune system in an attempt to provide valuable information about new molecules responsible for triggering death signals, such as galectin-1. The implications of this protein will be discussed in the context of T cell physiology and the regulation of central and peripheral tolerance. Finally, novel and intriguing findings will also be discussed implicating the use of this carbohydrate-binding protein in the treatment of autoimmune and inflammatory diseases.

\* Addressed correspondence: Dra. Claudia E Sotomayor, Laboratorio de Inmunología. Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Ciudad Universitaria. (5000) Córdoba. Argentina. Phone # 54-0351-4334164. Fax # 54-0351-4334187. E-mail: csotomay@bioclin.fcq.unc.edu.ar

† Both authors contributed equally to this article.

## **APOPTOSIS: A PHYSIOLOGICAL MECHANISM OF HOMEOSTASIS AND SELF-TOLERANCE IN THE IMMUNE SYSTEM**

Physiological cell death by activation of intrinsic cell suicide program, provides an efficient and critical control point for eliminating unwanted cells. This type of regulation allows the elimination of cells that have been produced in excess, have developed improperly, or have sustained genetic damage (Schwartzman and Cidlowski, 1993, Thompson, 1995). As to the initiation of the death program, the decision of a cell to undergo apoptosis can be influenced by a wide variety of extrinsic and intrinsic regulatory stimuli (Thompson, 1995). On the other hand, the effector stage takes place after triggering a number of evolutionarily conserved genes that regulate a final common cell death pathway, that is preserved from invertebrates to humans (Vaux et al., 1994; Raff, 1992).

Programmed cell death is an important physiological process acting both during development and homeostasis. Aberrations in this process are implicated in a broad range of diseases. While loss of apoptotic response can lead to cancer or autoimmune diseases, an increased apoptotic rate is implicated in neurodegenerative diseases, brain ischaemia and myocardial infarction. In this context, the immune system offers an excellent scenario for the discussion of the relevance of apoptosis in the development and maintenance of homeostasis. Immunologists have focussed largely on defining the stimuli that induce growth, differentiation and effector functions of lymphocytes and over the last two decades, the essential feature of lymphocyte activation and immune responses have been defined in considerable detail. Less is known, however, about the mechanisms that terminate immune responses. Mechanisms such as apoptosis are important after a productive immune response to a foreign antigen, when the immune system is returned to a state of rest (van Parijs and Abbas, 1998). This process allows the immune system to respond effectively to a new antigenic challenge. Moreover, apoptosis is crucial for achieving

self-tolerance avoiding the development of receptors capable of recognizing self-antigens. Elucidating the nature of these homeostatic mechanisms may lead to better strategies for suppressing harmful immune responses and for augmenting and sustaining beneficial responses to microbial vaccines and tumors.

### **Death Signals in T-lymphocytes Development**

The development of T cells is governed by the micro-environment of the thymus. A large number of precursor cells migrate into the thymus daily, where they are subjected to selection in a critical process of thymic education. Distinct stages of the T cell differentiation and maturation within the thymus have been identified and associated with the cells surface expression of molecules such as CD4, CD8 and the TCR/CD3 complex. In the subset of CD4<sup>+</sup> CD8<sup>+</sup> double-positive (DP) lymphocytes, more than 95% of cells are destined to die by positive and negative selection. The majority of DP thymocytes fail to generate a functional TCR that successfully interacts with major histocompatibility complex proteins (MHC) and consequently die by a process called death by neglect. Those cells bearing TCR that recognize MHC with intermediated affinity are positively selected. Moreover, a subset of DP cells recognizing MHC molecules with high affinity are subjected to negative selection and consequently deleted by apoptosis (Surh and Sprent, 1994). A high percentage of the thymocytes generated daily die within the thymus. Hence, massive cell death is a crucial part of T-cell development, as reflected by the fact that each new T cell undergoes for self-MHC restriction and self-tolerance. Thymocytes that successfully pass through positive and negative selections down-regulated the expression of either CD4 or CD8 and differentiate into functional single-positive cells and go out as mature T cells to the periphery.

Apoptosis in DP thymocytes is also triggered by glucocorticoids (Surh and Sprent, 1994). The induction of cell death in thymocytes by glucocorticoids was one of the first systems studied by Wyllie (1980) and Cohen et al. (1992). This work provides an early model of cell death by a specific signaling molecule

and was fundamental to the development of the concept of active cell death as a cellular response rather than a passive act of overwhelming cell damage. Thymocytes, in addition to T-cells lines, are highly sensitive to apoptosis induced by exposure to endogenous glucocorticoids (Sprent et al., 1988) in an active process, requiring *de novo* gene expression (Wyllie, 1980). In this sense, Ashwell and colleagues performed *in vivo* experiments (Vacchio et al., 1994, King et al., 1995) demonstrating that a subset of thymic epithelial cells are steroidogenic and that inhibition of steroid synthesis modified the profile of lymphoid thymic populations. Furthermore, data obtained by the creation of transgenic lines of mice carrying a glucocorticoid receptor antisense (King et al., 1995), indicated that thymocytes that do not bind to MHC are deleted by endogenous steroids.

Elimination of autorreactive lymphocytes may occur via activation-induced cell death: the same signals that trigger activation of peripheral mature T cells induce apoptosis of thymocytes (Takayama and Sitkovsky, 1989). The difference between positive selection of normally functional immature T cells and elimination of autorreactive cells may lie in the affinity of their TCRs for self antigens and increasing evidence suggest that co-stimulatory signals could play an important role in this process (Takayama and Sitkovsky, 1989, Iwatu et al., 1992, Migliorati et al., 1992).

In the network of intrathymic signals, the positive selection might also result from antagonism between glucocorticoid and activation-induced death. This model clearly demonstrates that thymocytes unable to bind to the MHC are eliminated via glucocorticoid signals, those cells that engage T cell receptor complex with moderate avidity are protected via the steroid/ TCR antagonism, whereas thymocytes able to transduce TCR signals of sufficient strength, are eliminated via activation-induced cell death.

### **The Fas Death Factor: "Turning Mature Lymphocytes Off"**

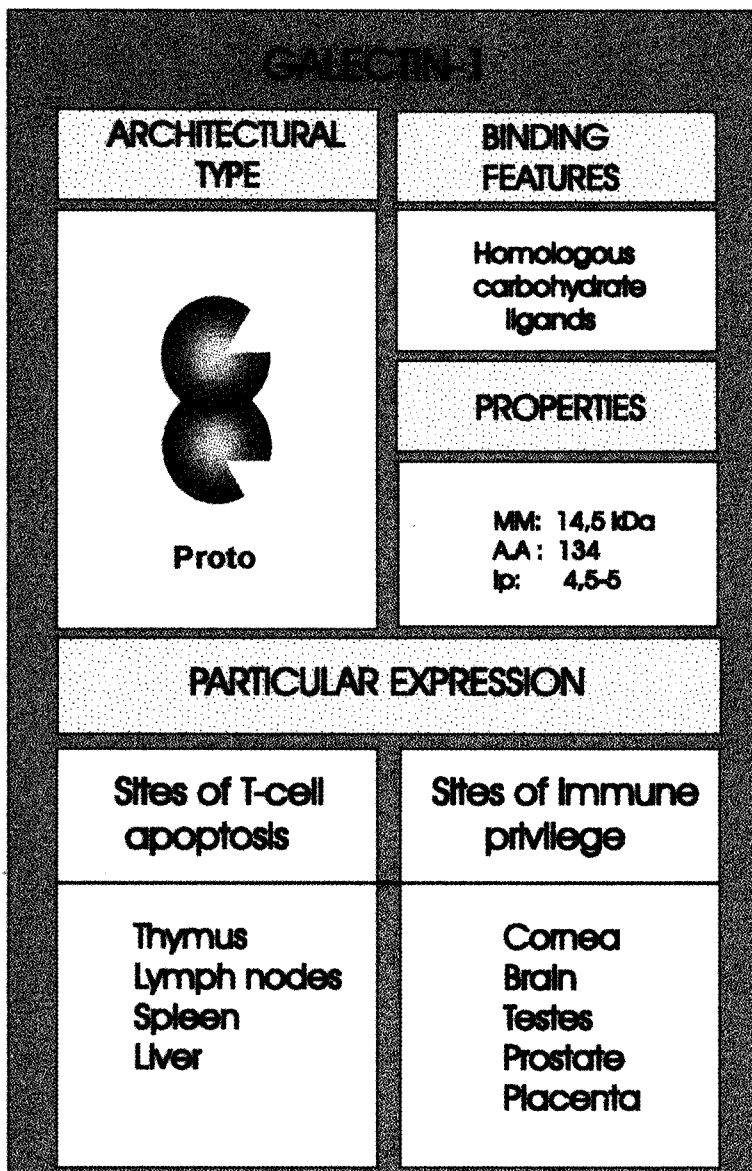
Among the most important molecules involved in triggering apoptosis appears the cell surface receptor Fas, also called APO-1 or CD95, which induced

apoptosis upon binding to its natural ligand FasL, APO-1L, or CD95L, or specific agonist antibodies (Nagata, 1997). This death receptor is a member of the tumor necrosis factor (TNF) and nerve growth factor (NGF) receptor superfamily, which among others, also includes TNF-R1, TNF-R2, low-affinity NGFR, CD40 and CD30. While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, CD95 and TNF-R1 also share an intracellular region of homology, designated the "death domain", which is required to signal apoptosis.

Fas, a 36 kDa type I membrane protein, is expressed on a wide variety of cell types including hematopoietic and epithelial cells. Expression of Fas on T and B-lymphocytes increases after antigen receptor-mediated activation (Nishimura et al., 1995). This molecule is also expressed in cells transformed with human T-cell leukaemia virus (HTLV-1), human immunodeficiency virus (HIV) or Epstein-Barr virus (EBV). Many nonlymphoid tissues, such as liver, also express Fas (Nagata, 1997; Galle et al., 1995).

Fas-L is a 40 kDa type II transmembrane protein that mediates apoptosis by crosslinking of Fas in sensitive target cells and in contrast to the widespread distribution of Fas, its ligand exhibits a highly restricted pattern of expression. FasL is induced on mature CD4+ and CD8+ T lymphocytes following activation, but it is not expressed by other hematopoietic cells (Tanaka et al., 1995; Suda et al., 1995). This molecule has been shown to play a role in the maintenance of peripheral T- and B-cell homeostasis. Noteworthy, in some circumstances FasL can be proteolytically cleaved from the membrane by a metalloproteinase, occurring in a soluble form that can act as a cytotoxic effector molecule or an inhibitor of apoptosis (Strand and Galle, 1998).

The Fas-mediated apoptotic cascade is initiated by the direct association of the death receptor Fas with the adapter molecule FADD (Fas-associated protein with death domain) and the effector protease FLICE (FADD homologous ICE/ CED-3-like protease, FADD-like ICE), a member of the ICE/ Ced-3/ caspase family (Henkart, 1996). The assembly of this signalling complex triggers the caspase cascade. The



MM: Molecular mass, A.A: Amino acid residues, Ip: Isoelectric point

FIGURE 1 Galectin-1, characteristic and particular expression

activated caspase members can cleave various substrates resulting in characteristic apoptotic morphology of cytoplasm and nuclei.

Activated T-cells express Fas as well as FasL. Although they use FasL to kill their targets, they can also use this molecular weapon against each other to

limit their own number protecting activated mature T cells from continued secretion of potentially harmful levels of cytokines. These cells are eliminated from the circulation by activation of the cell death program (Russel et al., 1993; Singer and Abbas, 1994).

### **Role of Fas-mediated cell death in the suppression of immune response, self-tolerance and autoimmunity**

Activation-induced apoptosis of mature T cells occurs via Fas and Fas ligand (FasL) interactions. A set of *in vitro* experiences using cell lines or T-cells hybrids provided successful information about the relevance of Fas death signals (Bruner et al., 1995; Dhein et al., 1995; Ju et al., 1995). In the recent years, much work has focused on the molecular mechanisms by which these molecules regulate apoptosis specially the relationship of Fas-FasL interaction with members of the Bcl-2 family in the context of peripheral immune tolerance (van Parijs et al., 1998).

van Parijs and colleagues clearly demonstrated that Bcl-2 protects T cells from apoptosis caused by the absence of growth factors and activation stimuli, a process called passive cell death and prolongs the response of T cells to a model of foreign antigen *in vivo*. In contrast, Fas induces apoptosis in autorreactive T cells or in activated T cells that are restimulated with high concentrations of antigen by a process called activation-induced cell death. *In vivo*, Fas-mediated apoptosis is responsible for eliminating T cells responding to a model systemic self-antigen and for preventing autorreactive helper T cells from activating self-reactive B cells.

Genetic defects that predispose to autoimmunity are providing valuable information about the mechanisms responsible for terminating T-cell responses to self-antigens. In this sense, the importance of Fas/FasL interaction in peripheral tolerance, has been highlighted by the MRL-*lpr/lpr* or C3H-*gld/gld* mice strains which carry spontaneous mutations in Fas and FasL genes respectively. These mice exhibit multiple autoimmune systemic disorders characterized by the presence of autoantibodies, hypergammaglobulinemia and immune complex nephritis, all features of a lupus-like syndrome (Russell et al., 1993; Russell and Wang, 1993). Nagata and colleagues (Adachi et al., 1995) created a Fas<sup>-/-</sup> mice by targeted deletion of the Fas gene. These mice displayed enhanced and accelerated lymphoproliferation in comparison to *lpr/lpr* mice (Adachi et al., 1995).

Recently, a human syndrome of autoimmunity associated lymphadenopathy has been described, carrying various inherited abnormalities in Fas-mediated killing. These abnormalities include the inheritance of two mutant Fas alleles and unknown signalling defects (Fisher et al., 1995; Rieux-Laucat et al., 1995). It is likely that other alteration in Fas or downstream signalling intermediates will be identified as cause of autoimmune syndromes. Recently, an inherited human caspase 10 mutation has been described showing defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome (type II) (Wang et al., 1999).

The elucidation of the mechanisms involved in T cell survival *in vivo* will lead to rational approaches for controlling autorreactivity, while enhancing immunological memory.

### **Role of Fas-mediated cell death in immunological privileged tissues**

The immune privileged tissues are vulnerable sites in the body where even minor cellular immune reactions and their associated inflammatory response can cause irreversible damage. Therefore, protective mechanisms are required to avoid unwanted immune reactions that could result in impaired organ functions. Interestingly, not only are these "immune privileged sites" protected against overwhelming inflammatory responses, but they can also support allogenic or xenogenic tissue grafts. Some explanation about how immune privileged is maintained, involve physical barriers and cytokines profiles (Streilin, 1993).

FasL has been also reported to be constitutively expressed in two immunologically privileged tissues, such as the eye and the testis. Griffith et al. (1995) showed that the constitutive expression of FasL on parenchymal cells within the anterior chamber of the eyes can maintain the integrity of this immune-privileged site. It can be reported that Fas<sup>+</sup> lymphoma cells can be induced to undergo apoptosis when exposed *in vitro* to explants of cornea and iris-ciliary body from eyes of normal mice, but not when exposed to eyes of *gld* mice, which do not express FasL. Interestingly, FasL has been found to be

expressed in Sertoli cells of the testis (Bellgrau, 1995).

Taken together, these findings unequivocally suggest that FasL plays a crucial role by avoiding the damage inflicted by activated T cells to these tissues. The expression of high levels of FasL represents a defensive mechanism to prevent damage caused by inflammation through an induction of apoptosis of activated cells expressing elevated levels of Fas antigen (Osborne, 1996).

Recent data confirm that expression of functional FasL might confer the status of immune privilege to tumor cells, representing an active defense mechanism resulting in the elimination of immune competent anti-tumor lymphocytes (O'Connell et al., 1999). In this sense, FasL expression could be associated with the later tumor recurrence which is commonly observed in several tumors such as melanomas. These tumors often recur after 20 years or more, and this can be explained by a loss of immunosurveillance. Hence, FasL expression might contribute to tumor progression, invasion or metastasis.

## **GALECTINS: A FAMILY OF CARBOHYDRATE-BINDING PROTEINS WITH IMMUNOREGULATORY PROPERTIES**

### **Definition and Background**

Galectins are a growing family of animal  $\beta$ -galactoside binding proteins, defined by two common characteristics: (a) affinity for poly-*N*-acetylglucosamine-enriched glycoconjugates and (b) significant sequence homology in the carbohydrate binding site (Barondes et al., 1994a; Barondes et al., 1994b). In the past few years, there has been progress in identifying new galectins in mammals and other species, cloning them and ascertaining the structural features that determine carbohydrate binding. Ten mammalian galectins have been well characterized and studied (Leffler, 1997). Structural analyses of various galectins indicate the presence of homodimers of carbohydrate-binding domains in galectin-1 and galec-

tin-2, a monomer of the carbohydrate-binding domain in galectin-5 and a single polypeptide chain with two carbohydrate-binding domains joined by a link peptide in galectins-4, -6, -8 and -9. Galectin-3 has a carbohydrate-binding domain, a short N-terminal segment, consisting of PGAYPG (X) repeats and an intervening stretch of amino acids, enriched with proline, glycine and tyrosine. Expression analysis have revealed that certain galectins display a restricted distribution, e.g. galectin-2 in hepatoma, galectin-4 in small intestine, galectin-5 in erythrocytes and galectin-7 in keratinocytes. Galectins with a broad tissue distribution include galectin-1, expressed in cardiac, smooth and skeletal muscle, macrophages, neurons, thymus, kidney and placenta, galectin-3 present in blood cells such as monocytes, mast cells, and tumor cells and galectin-8 expressed in liver, kidney, cardiac muscle, lung and brain (Rabinovich, 1999). Extensively studied among them is galectin-1, an homodimer with an Mr of approximately 14,500 Da. It has been postulated that this protein recognizes a wide variety of extracellular receptors such as fibronectin (Ozeki et al., 1995) and laminin (Zhou and Cummings, 1993) and cell surface glycoproteins such as CD45 and CD43 (Baum et al., 1995a, Perillo et al., 1995) through deciphering specific glyco-codes (Kasai and Hirabayashi, 1996).

By virtue of this specific recognition, this evolutionarily conserved family of animal lectins have been implicated in a variety of functions that include cell growth regulation (Sandford and Harris-Hooker, 1990; Wells and Mallucci, 1991), cell adhesion (Cooper et al., 1991; Zhou and Cummings, 1993; Rabinovich et al., 1999a), neoplastic transformation (Akahani et al., 1997a), immune responses (Ofner et al., 1990; Levy et al., 1983) and T-cell apoptosis (Perillo et al., 1995; Rabinovich et al., 1998; Iglesias et al., 1998a). However, the widespread expression of multiple members of the galectin family and presumed overlaps in carbohydrate-binding specificities have made it difficult to establish the *in vivo* function of individual members of this class of proteins (Poirier and Robertson, 1993).

All known members of this family lack a signal peptide (Barondes et al., 1994a), are found in the

cytosol and are isolated as soluble proteins. However, there is evidence that some members are externalized by an atypical secretory mechanism (Cooper and Barondes, 1990).

The expression pattern of different galectins changes during development (Colnot et al., 1997) and this pattern is also altered at sites of inflammation and in breast, colon, prostate and thyroid carcinomas (Akahani, et al. 1997b). The level of expression of some galectins by tumor cells has been shown to be correlated with metastatic potential. Although galectins exert their effects through recognition of a spectrum of appropriately glycosylated proteins on the surface of a variety of cells, the precise mechanism and signal transduction pathways involved in these functions remain largely unknown.

#### **GALECTIN-1: EXPRESSION WITHIN THE IMMUNE SYSTEM AND IMPLICATIONS IN T-CELL PHYSIOLOGY**

##### **Participation of Galectin-1 as a Gear of the Central and Peripheral Cell Death Machinery**

Galectin-1 has been shown to be expressed in sites where T-cell apoptosis takes place including the thymus (Baum et al., 1995a), spleen (Rabinovich et al., 1996) and lymph nodes (Baum, et al., 1995b). It has been particularly found in thymic epithelial cells (Baum et al., 1995a), activated macrophages (Rabinovich, et al., 1998) and effector T cells (Blaser, et al., 1998) (Figure 1).

The first evidence suggesting that galectin-1 could be involved in central immune tolerance was first suggested by Goldstone and Lavin (1991), who reported an increase in the levels of galectin-1 mRNA during apoptosis induced by glucocorticoids. As clearly stated, the interplay between thymic steroids and TCR signals modulate cell death within the thymus (Wyllie, 1980). It is well known that thymocyte maturation also requires the participation thymic epithelial cells and extracellular matrix components (Anderson et al., 1994; Anderson et al., 1996). In this sense, Baum et al. (1995a) demonstrated that human

thymic epithelial (TE) cells produced high levels of galectin-1 which bound specifically to the surface of cortical thymocytes. This endogenous lectin mediated the adhesion of thymocytes to TE cells. Sensitivity of T cells to galectin-1 was found to be modulated by the expression of glycosyltransferase enzymes that might modify the availability of oligosaccharide ligands for galectin-1. Perillo et al (1997) provided then concluding evidence that galectin-1 induced apoptosis of two distinct subpopulations of non-selected and negatively-selected CD4<sup>low</sup>, CD8<sup>low</sup> immature cortical thymocytes (Perillo et al., 1997). Null mutant mice in galectin-1 gene will be useful to confirm whether galectin-1 plays a critical role in the central cell death machinery for positive and negative selection of developing thymocytes.

Activation-induced cell death of mature T cells is one of the mechanisms aimed at turning off the immune response and preventing the expansion of autoaggressive clones. In addition to its role in central tolerance, Perillo et al. (1995) clearly showed that galectin-1 induced apoptosis also in activated mature T cells. Recently, Blaser et al. (1998) found that galectin-1 expression was strongly up-regulated in effector T cells and inhibited antigen-induced proliferation of naive and memory CD8<sup>+</sup> T cells. This mechanism was mediated by an arrest in cell cycle progression at the level of S and G2/M stages (Allione et al., 1998).

Moreover, we have recently shown the presence of a galectin-1-like protein, which was differentially regulated in resident, inflammatory and activated macrophages (Rabinovich et al., 1996). Total and surface expression of this carbohydrate-binding protein, called RMGal (for rat macrophage galectin-1) were found to be up-regulated when these cells were activated with protein kinase C activators such as phorbol esters (PMA) and chemotactic peptides (fMLP). When this protein was purified by affinity chromatography and its biochemical properties and amino acid sequence were determined, a definitive conclusion was reached concerning its pertinence to the galectin-1 subfamily (Rabinovich et al., 1998).

Macrophages play an important role in several steps of innate and adaptive immune response. While

they have great phagocytic ability and a large repertoire of lytic enzymes and secretory products, they also express a wide array of cytokines, surface receptors able to recognize specific antigen epitopes. Activated macrophages are more efficient in their ability to process and present antigens in the initiation of an immune response by virtue of the higher levels of major histocompatibility complex molecules (Adams and Hamilton, 1984; Adams et al. 1996). Moreover, they are also key immunoregulatory cells able to turn off an established immune response (Aliprantis et al., 1996). We determined that by using current techniques to evaluate apoptosis, such as DNA fragmentation, TUNEL assay and transmission electron microscopy that galectin-1 produced by activated macrophages is able to induce apoptosis of mature T cells in a carbohydrate-dependent manner (Rabinovich et al., 1998). The results were comparatively stronger to those found in an heterologous system using CLL-I, the 16 kDa chicken isolectin (Rabinovich et al., 1997).

RMGal protein was found to be secreted only when macrophages were activated with potent biochemical agents and pro-inflammatory cytokines (Rabinovich et al., 1999c).

#### **Galectin-1 Expression in Immune Privileged Sites: a Novel Mechanism of Protection?**

Galectin-1 is also present in sites of immune privilege, such as placenta (Hirabayashi and Kasai, 1988; Iglesias et al., 1998a), cornea (Ogden et al., 1998) and prostate (Allen et al., 1991; Hirabayashi and Kasai, 1993). The presence of this protein in these vulnerable sites might contribute to maintain a state of tolerance by inducing apoptosis of inflammatory and activated T cells that could provoke injury, autoimmune damage or infection. Accordingly, galectin-1 could also be proposed as an alternative regulatory signal to regulate immune privilege. Expression of this protein in first term gestation placenta would prevent inflammatory T cells from harming the fetus (Iglesias et al., 1998a). In agreement, a protein related to the galectin family called GRIFIN (galectin-related interfiber protein) has been recently

identified in lens, cellular structures of the optical system (Ogden et al. 1998). Furthermore, recent results reported by Maldonado et al. (1999) have clearly shown by using immunogold techniques, that galectin-1 is expressed in Müller cells in post-natal chicken retina and in mitochondria localized in the inner segments of cone cells. Expression of this protein in these glial cells suggests a potential role in metabolic and immunomodulatory processes between Müller and other retinal cells.

This pro-apoptotic protein was found to be up-regulated by metastatic in comparison to non-invasive tumors. In certain way, tumors might be considered as immune privileged sites and several mechanisms for tumor evasion of immune recognition have been proposed, such as decreased expression of MHC class I or B7.1 co-stimulatory signal, TGF- $\beta$  secretion, endocytosis of tumor antigens and FasL expression (O'Connell et al., 1999). In this sense, one should suspect that galectins in tumor cells can trigger apoptosis of tumor-infiltrating lymphocytes (TILs), thus allowing the tumor to escape immune attack.

#### **Death Signals in the Periphery**

Despite striking similarities in their localization, critical differences should be distinguished between FasL and galectin-1. FasL induces apoptosis by a interaction with its counterpart Fas/APO-1/CD95 within the same cell (suicide) or a neighbour cell (fratricide), while galectin-1 is secreted and binds to cell surface glycoconjugates (Perillo et al., 1997) on cortical thymocytes and T cells. Besides, galectin-1 and FasL apparently use different signal transduction pathways to engage the apoptotic program of the cell. Recently, Su et al. (1996) and Perillo et al. (1995) showed that the T lymphoblastoid cell line MOLT-4 that was insensitive to FasL-induced apoptosis, was susceptible to galectin-1. In contrast, the T lymphoblastoid cell line CEM, which was sensitive to FasL was resistant to galectin-1-induced apoptosis. These data strongly suggest that galectin-1-induced apoptosis are clearly distinct from those triggered by Fas engagement.

About the apoptotic signal trigger by cross-linking the T-cell receptor complex, experiments performed



whit galectin-1 and T lymphoblastoid cell line that not express CD3, demonstrate that the lectin is capable to activated the death cell program (Pace and Baum, 1997). These results suggest that the mechanism by which galectin-1 can induced apoptosis appears to be distinct from T cell receptor trigger apoptosis.

### **Death vs proliferation: Galectin-1 vs Galectin-3**

While galectin-1 has been shown to trigger T cell apoptosis (Perillo et al., 1995; Rabinovich et al., 1998; Iglesias et al., 1998a), galectin-3 has been shown to stimulate proliferation (Yang et al., 1996; Iglesias et al., 1998b; Inohara et al., 1998). Similarly to members of the Bcl-2 family, galectins-1 and -3 belong to an additional family of proteins with high sequence homologies but opposite effects on cell survival. The balance between the competing activities of pro-apoptotic proteins such as Bax, Bad and Bak and on the other hand anti-apoptotic proteins such as Bcl-2 and Bcl-xL, determines cell fate (Adams and Cory, 1998). Proteins most similar to Bcl-2 promote cell survival by inhibiting adapters needed for activation of the proteases (caspases) that dismantle the cell, while more distant relatives instead promote apoptosis apparently through mechanisms that include displacing the adapters from the pro-survival proteins. In this sense, the family of Bcl-2 related proteins constitute one of the most relevant apoptotic regulatory gene products acting at the effector stage of apoptosis (Kröemmer, 1997). Hence, it seems meaningful that the interplay between galectins-1 and -3 could also represent an alternative pathway in the normal control of cell homeostasis. To support this hypothesis a striking homology has been found between galectin-3 and Bcl-2 particularly localized in the NWGR domain (Yang, 1996; Akahani et al., 1997).

### **Galectin-1 in T cell Adhesion to Extracellular Matrix**

Despite the lack of a secretion signal sequence, galectin-1 is secreted into the extracellular milieu, where it

recognizes poly-N-acetyl-lactosamine chains on major ECM components, such as laminin (Zho and Cummings, 1993) and fibronectin (Ozeki et al., 1995). By virtue of this recognition, this carbohydrate binding protein has been suggested to act as a modulator of cell-cell and cell-ECM interactions. In collaboration with the laboratory of Dr. Ofer Lider in the Weizmann Institute of Science, Israel, Rabinovich et al. (1999a) has recently shown that galectin-1 (at concentrations below its apoptotic threshold) inhibited the adhesion of human T cells to ECM glycoproteins in a dose and carbohydrate-dependent manner. The inhibition of T-cell adhesion correlated with the ability of this protein to block the re-organization of cell's actin cytoskeleton. Finally, the production of pro-inflammatory cytokines in the context of the ECM was markedly reduced in the presence of this carbohydrate-binding protein. This is the first report as to the role of galectin-1 in T cell adhesion. However, this protein has been shown to promote cell attachment or detachment on other cell systems such as myoblasts (Cooper et al., 1991), melanocytes (van den Brulle et al., 1995), olfactory neurons (Mahantahappa et al 1994), rhabdomyosarcoma cells (Ozeki et al, 1995) and fibroblasts (Zhou and Cummings, 1993).

## **GALECTIN-1 IN T-CELL IMMUNOPATHOLOGY**

### **Galectin-1, Programmed Cell Death and Autoimmunity: an Attractive Association**

Autoimmune disease challenges clinical immunology to set the system right. An autoimmune disease is caused, according to the clonal selection paradigm by aberrant activation of the immune response and loss of central and/or peripheral immune tolerance to self-antigens (Cohen, 1995). The rational answer for harmful activation is to find a way to deactivate the pathogenic lymphocytes. As aforementioned, observations in murine models of systemic autoimmunity and in Canale-Smith syndrome suggest that regula-

tion of lymphocyte apoptosis is crucial to the maintenance of peripheral tolerance (Singer et al., 1994; Fisher et al., 1995). In this context, one should expect that knock out mice for galectin-1 would evidence autoimmune manifestations, such as lupus-like disorders or arthritis, as observed for spontaneous mutations in Fas and FasL in *lpr/lpr* or *gld/gld* mice respectively. However, no important phenotypic changes could be detected in null-mutant mice as regards galectin-1 gene (Poirrier and Robertson, 1993). An exhaustive examination of the immunological system is imperative in these genetically modified mice not only at the central level but also at the periphery to search for potentially harmful autoaggressive clones and signs of disregulated apoptosis.

Implications of galectin-1 in central and peripheral immune tolerance prompted us to investigate its therapeutic potential in collagen-type II-induced arthritis (CIA) in DBA/1 mice, an experimental model of rheumatoid arthritis (Durie et al., 1994). In collaboration with the laboratory of Dr. Chernajovsky in London, Rabinovich et al. demonstrated by using gene and protein therapy strategies that galectin-1 suppressed arthritis via T cell apoptosis (Rabinovich et al., 1999b). A single injection of syngenic DBA/1 fibroblast engineered to secrete galectin-1 at the day of the disease onset, as well as daily administration of recombinant galectin-1, were both able to abrogate clinical and histopathological manifestations of arthritis. Both treatments resulted in the inhibition of anti-collagen type II (C-II) antibody levels, inhibition of the pro-inflammatory response and a shift towards a Th2-mediated immune response, as judged by the anti-CII IgG isotypes in mice sera at the end of the treatment and the cytokine profile in draining lymph node cells. Finally, clear-cut evidence was provided to show that mice engaged in the gene therapy protocol with galectin-1 increased their susceptibility to antigen-induced apoptosis, providing the first correlation between the apoptotic properties of galectin-1 and its therapeutic potential *in vivo*.

Rheumatoid arthritis (RA) is a common chronic autoimmune disease for which there is not effective therapy capable of preventing long-term progression and joint damage (Feldmann et al., 1996; Chernajo-

vsky et al., 1995). Therefore, effective treatment of arthritis will require the elimination of arthritogenic lymphocytes that initiate and perpetuate joint inflammation, as well as the induction of tissue repair. Hence galectin-1-induced apoptosis could provide for an ideal mechanism using a naturally occurring protein to terminate the autoimmune T-cell attack, preventing the expansion of dominant autoaggressive clones (Vaishnav et al., 1997). It has been clearly suggested that the extent of apoptosis in RA is inadequate to counteract ongoing proliferation. This imbalance may be explained by the production of cytokines such as IL-1 $\beta$ , which favor synoviocyte and T-cell proliferation and inhibit susceptibility to apoptosis, possibly associated with increased expression of the Bcl-2 family of proteins (Tsuboi et al., 1996). TNF- $\alpha$  which acts as a potent pro-inflammatory molecule in RA, signals predominantly through the nuclear factor kappa B (NF $\kappa$ B) pathway, promoting the expression of adhesion molecules and recruiting additional cytokines such as GM-CSF and IL-6 in the inflamed joint. Signaling through NF- $\kappa$ B has been suggested to inhibit apoptosis (Fujisawa et al., 1996). Finally, an increase in soluble truncated Fas has been detected in RA synovial fluid thus inhibiting the functional interaction between Fas and FasL (Hasunuma et al., 1997). Altogether, these findings suggest that a disregulated activation of programmed cell death is a critical component of the ethiopathogeny of RA.

Results concerning the role of galectin-1 in suppressing an autoimmune inflammatory process are in agreement with those raised by Levy et al. (1983) in a model experimental autoimmune myasthenia gravis in rabbits and those raised by Offner et al. (1990) in experimental autoimmune encephalomyelitis in Lewis rats.

## CONCLUDING REMARKS

The elucidation of the biochemical pathways and specific proteins that regulate programmed cell death provide a remarkable opportunity to manipulate the life-and death decisions of the cells. The basic understanding and therapeutic manipulation of pro-

grammed cell death will have far-reaching implications for the future health of autoimmune disease patients. In this sense, galectins represent an attractive target for biomedical research and clinical intervention. Experimental evidence is now emerging to support the use of galectin-1 not only in the treatment of autoimmune disease, but also in medical strategies aimed at targeting T-cell physiopathology such as the inhibition of transplant rejection, control of graft versus host disease and inhibition of chronic inflammatory processes.

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