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HOW DOES IT ACT WHEN SOLUBLE? CRITICAL EVALUATION OF MECHANISM OF GALECTIN-1 INDUCED T-CELL APOPTOSIS

SHORT COMMUNICATION

Andrea Blaskó, Roberta Fajka-Boja, Gabriela Ion and Éva Monostori*

Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

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Galectin-1 (Gal-1), a mammalian lectin induces apoptosis of T lymphocytes. Contradictory data have resulted in confusing knowledge regarding mechanism of Gal-1 induced T-cell apoptosis. In this paper we aimed to resolve this controversy by comparing cell death induced by low (1.8 μM, lowGal-1) and high (18 μM, highGal-1) concentration of soluble Gal-1. We show that lowGal-1 and highGal-1 trigger phosphatidylserine exposure, generation of rafts and mitochondrial membrane depolarization. In contrast, lowGal-1 but not highGal-1 is dependent on the presence of $p56^{lck}$ and ZAP70 and activates caspase cascade. The results allow the conclusion that the cell-death mechanism strictly depends on the concentration of Gal-1.

Keywords: Galectin-1 – apoptosis mechanism – concentration dependence

Galectin-1 (Gal-1), a member of the β-galactoside binding galectin superfamily plays role in regulation of viability of activated peripheral T lymphocytes and tumor T-cell lines [7, 9], hence it contributes to the immunological balance in physiological and pathological situations [16]. Data describing the mechanism of Gal-1 induced T-cell apoptosis have been obtained using soluble, recombinant Gal-1 and remained highly controversial [1, 5, 7, 9, 10, 19]. The receptor tyrosine phosphatase CD45, initially described as apoptotic receptor for Gal-1 [14, 15, 23] has recently been shown to be dispensable [4, 11, 12]. Gal-1 treatment induces partial TCRζ chain phosphorylation, generating pp21ζ and limited receptor clustering at the TCR contact site [22] and hence it antagonizes with the TCR signal transduction and promotes apoptosis. Accordingly we have recently described the crucial function of $p56^{lck}$ and ZAP70 in Gal-1 induced T cell apoptosis [8, 9]. Our previous data [7–9] and those provided by other laboratories [1, 10, 21] support that it is a mitochondrial type of caspase-mediated cell death, however other researchers have shown that Gal-1 triggers a mitochondrial route independent of the caspase cascade [5]. Moreover, Stowell et al. [20] have published results indicating that Gal-1 prepares leukocytes for phagocytic elimination by inducing phosphatidylserine exposure on the outer surface of the plasma membrane without stimulating apoptosis.

*Corresponding author; e-mail: monos@brc.hu

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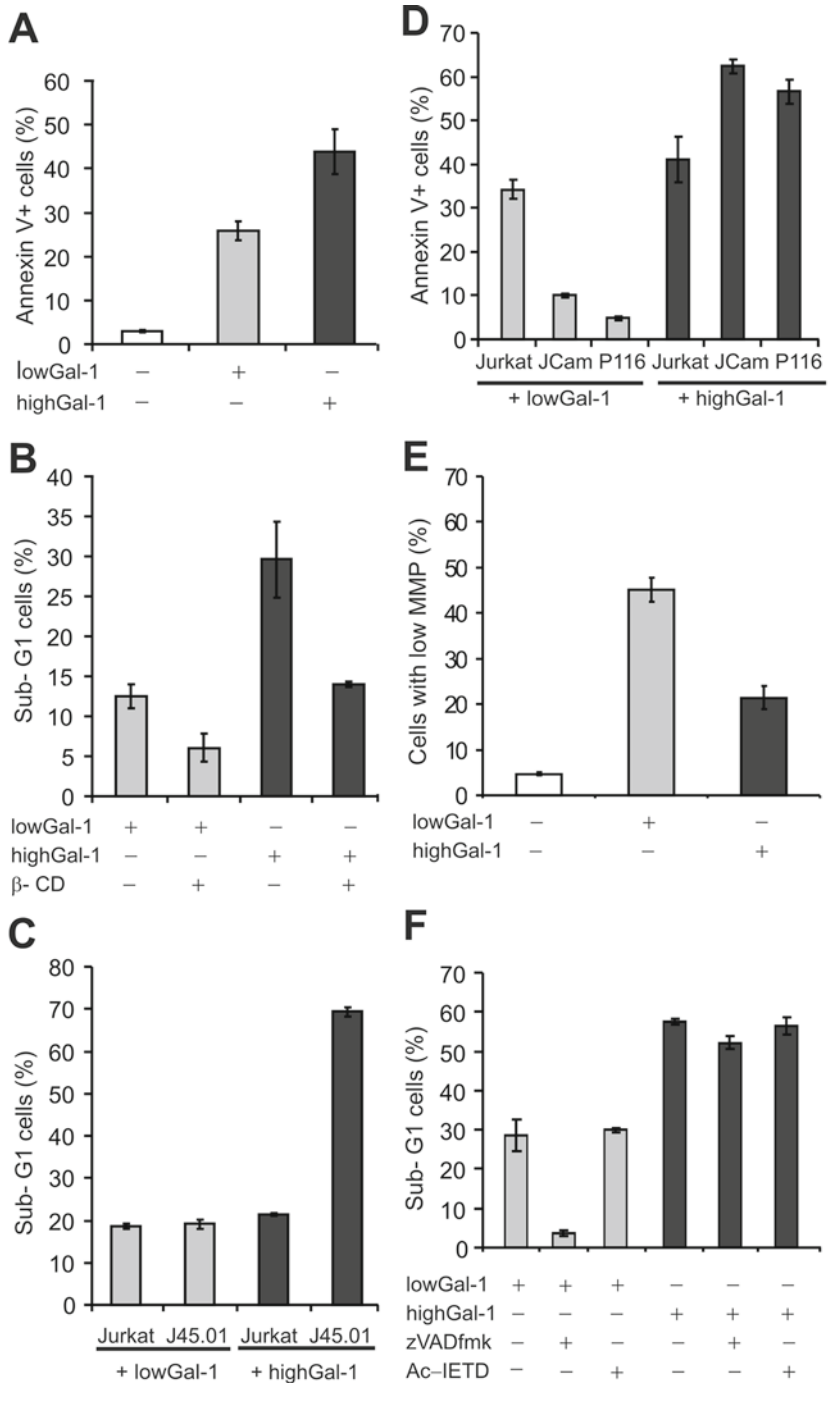
The root of these conflicts likely arises from the difference in the preparations of Gal-1 and concentration from 1 to 20 μM used in the assays. Lower and higher amounts of the protein might act on different transmitting receptors resulting in diverse apoptotic pathways. Therefore we aimed to carry out a systematic comparison of the T-cell apoptosis induced by low (1.8 μM, lowGal-1) and high (18 μM, high-Gal-1) concentration of soluble, recombinant Gal-1.

Both lowGal-1 and highGal-1 induced phosphatydilserine exposure from the inner to the outer surface of the plasma membrane (Fig. 1A). As it was previously shown membrane rafts were generated during Gal-1 mediated T-cell death [2, 7, 13]. Accordingly, inhibition of raft formation by β-cyclodextrin resulted in decrease of cell-death induced by either low or high concentration of Gal-1 (Fig. 1B). Requirement for the presence of CD45 as a functional Gal-1 receptor in apoptosis was indicated [14, 15, 23] and contra-indicated [4, 11, 12] in numerous papers. As it is shown in Fig. 1C Jurkat cells or CD45 deficient Jurkat cells committed suicide in the presence of lowGal-1 and highGal-1. Of note, deficiency in CD45 promoted highGal-1 triggered death of Jurkat T-cells. This finding was in accordance with a result of Nguyen et al. [11] determining as a negative regulator of the tyrosine phosphatase in this process. On the other hand, the expression of two T-cell specific non-receptor tyrosine kinases, $p56^{lck}$ and ZAP70 was necessary for apoptosis induction with low but not with high concentration of Gal-1 (Fig. 1D). Previous results from our [7, 9] and other laboratories [1, 5, 10] revealed that treatment of T-cells with Gal-1 triggered depolarization of the mitochondrial membrane. Indeed, this occurred when Jurkat cells were treated with low or high concentration of Gal-1 (Fig. 1E) confirming the existence of this pathway. Decrease of mitochondrial membrane potential could be followed with the activation of the caspase cascade as it was indicated in several papers [1, 7, 9, 10, 21] or a caspase independent pathway with release and activation of endonucleases as Hahn and co-workers suggested [5]. As it is presented in Fig. 1F, lowGal-1 but not highGal-1-induced apoptosis was inhibited with pan-caspase inhibitor, zVAD-fmk, validating the assumption that low concentration of Gal-1 caused caspase dependent while high amount of Gal-1 initiated caspase independent mitochondrial apoptotic pathway. None of the Gal-1 concentrations required the function of caspase 8, the initiator caspase for death receptor-mediated cell-death, since inhibitor of this caspase, Ac-IETD did not affect apoptosis (Fig. 1F).

The elaborate experimental comparison of T-cell apoptosis pathways stimulated with low or high concentration of Gal-1 demonstrated that depending on the amount of the used agonist different pathways were initiated. The question still remains open which apoptotic route represents the physiological one. One has to face difficulties when determining the physiological/pathological concentration of Gal-1 as this lectin occurs in negligible amount in soluble form in healthy and very low concentration in pathological serum [6, 18]. As Gal-1 remains bound to cell or extracellular matrix glycoconjugates, determination of the local concentration of Gal-1 is basically impossible. Moreover Gal-1 is a typical intracellular protein being secreted on a non-classical fashion [3]. From the apoptosis point of view only the extracellular Gal-1 is functional. Recombinant Gal-1 is always manipulated during purification and in


A
Annexin V+ cells (%)
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Annexin V+ cells (%)
Jurkat JCam P116 Jurkat JCam P116
+ lowGal-1
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B
Sub- G1 cells (%)
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E
Cells with low MMP (%)
lowGal-1
highGal-1
C
Sub- G1 cells (%)
Jurkat J45.01 Jurkat J45.01
+ lowGal-1
+ highGal-1
F
Sub- G1 cells (%)
lowGal-1
highGal-1
zVADfmk
Ac–IETD

←

Fig. 1. T-cell apoptosis induced by lowGal-1 and highGal-1

(**A**) Jurkat cells were stimulated with lowGal-1 (low concentration of recombinant protein, 1.8 μM) for 12 h or with highGal-1 (high concentration of recombinant protein, 18 μM) for 6 h. To determine phosphatidylserine (PS) exposure, cells were labeled with Annexin V–FITC (Pharmingen) for 15 min at room temperature and analyzed with cytofluorimetry (FACSCalibur, Becton and Dickinson). (**B**) For the analysis of raft generation, Jurkat cells were treated with or without lowGal-1 and highGal-1 for 24 h or 16 h in the absence or presence of 10 mM β-cyklodextrin (β-CD, Sigma). 'Sub-G1' cell population was determined by permeabilizing and staining the cells with 0.1% Triton X-100, 0.1% Na3 citrate, 10 μg/ml RNase and 10 μg/ml propidium iodide (Sigma) and analyzed with cytofluorimetry. (**C**) Jurkat and J45.01 (CD45 deficient Jurkat) cells stimulated with lowGal-1 for 24 or highGal-1 for 16 h were analyzed for 'Sub G1' cell population as described under (**B**). (**D**) Jurkat, JCam 1.6 ($p56^{lck}$-deficient Jurkat, JCam) and P116 (ZAP70-deficient Jurkat) cells were stimulated with lowGal-1 or highGal-1 and were analyzed for PS exposition as described under (**A**). (**E**) After 24 h or 12 h of Gal-1 treatment, cells were loaded with the mitochondrial membrane potencial (MMP) sensitive JC-1 (Fluka) for 15 min at 37 °C, and then the fluorescence intensity was measured by cytofluorimetry. (**F**) Jurkat cells were stimulated with lowGal-1 or highGal-1 for 24 h or 16 h, respectively, in the absence or presence of 50 μM zVAD-fmk (caspase inhibitor I, zVAD) or 50 μM Ac-IETD (caspase 8 inhibitor) (Calbiochem) then the sub-G1 cell population was analyzed as described under (**B**). Recombinant Gal-1 was produced and purified by lactose affinity chromatography as previously described [4]. See detailed description of the used methods in [7]

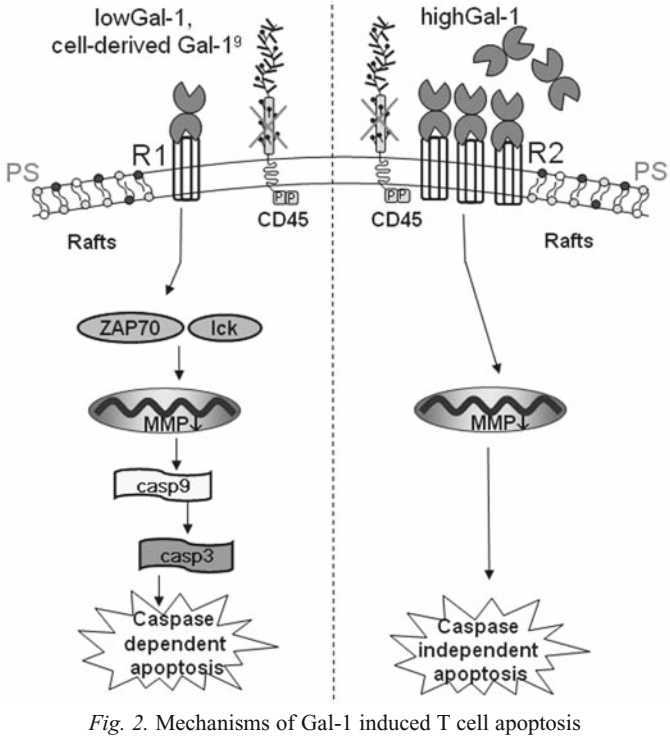

Fig. 2. Mechanisms of Gal-1 induced T cell apoptosis

Left panel: Cell-death triggered by low concentration of soluble and cell-derived Gal-1. Right panel: Apoptosis induced by high concentration of Gal-1. Abbreviations: R1 and R2: receptors 1 and 2; PS: phosphatidylserine; MMP: mitochondrial membrane potential; Casp9 and 3: caspases 9 and 3

apoptosis assays since it has to be in reduced form for functional conformation. To avoid this process we analyzed the role and mechanism of cell-derived Gal-1 in the apoptotic process [9]. In co-culture system Gal-1 remains as a native, functional protein without any chemical modification and the apoptosis assay also avoids addition of reducing agent. In Fig. 2 we summarized the major signaling components of Gal-1 induced apoptosis determined using soluble Gal-1 in low or high concentration and in the most physiological *ex vivo* co-culture system. Cell-derived Gal-1 and lowGal-1 drives Jurkat T-cells and activated peripheral T-cells [7, 9] to a caspase dependent mitochondrial route of apoptosis via unidentified receptor (Fig. 2. left panel, R1), in which $p56^{lck}$ and ZAP70 play essential role while highGal-1 stimulating unknown receptor (Fig. 2. right panel, R2) causes caspase independent mechanism of cell death without requirement of $p56^{lck}$ and ZAP70. These results strongly indicate that low lGal-1 induces the physiological mechanism of T-cell apoptosis. Although this comparison does not contain novel aspects of molecular components of Gal-1 induced T-cell death it contributes to understand the basic and valid pathway by the substantial and circumstantial comparison of Gal-1's apoptotic effect. This work is crucial due to the implication of Gal-1 in therapy of autoimmune/inflammatory diseases [17].

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