

PhD thesis

**Human immunosuppressive protein,
galectin-1 emerges as a novel regulatory
factor in autoimmune disorders**

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Introduction

Galectin-1 (Gal-1) is a member of a family of β -galactoside binding lectins with specific affinity to terminal N-acetyl-lactosamine motifs on multi-antennary cell surface N-glycans. It is partially inhibited by terminal α -2-3, and fully inhibited by α -2,6-sialylation. Gal-1 binding structures are present on almost all cell types, and participate in cell adhesion, migration, and signal transduction. Gal-1 has been implicated in both extracellular and intracellular roles. Its main function inside the cells is facilitation of mRNA splicing by associating with Gemin4. Extracellular Gal-1 has been known to cause apoptosis of T-cells. The lactosamine units required for galectin-1 binding and apoptosis are synthesized by specific glycosyltransferases and expression of such enzymes controls T-cell susceptibility to Gal-1 driven apoptosis. Gal-1 driven apoptosis requires the presence of Lck and ZAP70 kinases, release of ceramide, decrease of mitochondrial membrane potential and caspase activation.

Systemic lupus erythematosus (SLE) is one of the most common and devastating systemic autoimmune diseases. Molecular pathology in SLE is characterized by immune-mediated inflammation in multiple organs and by the production of autoantibodies. Malfunction of apoptosis is a major factor in SLE pathophysiology, with T-cell death occurring by necrosis.

Cell surface proteins carry oligosaccharide complexes synthesized through a series of enzymatic steps. Glycan complexes have been shown to play a role in adhesion, migration, and signal transduction into the cell. Protein glycosylation has become an integral part of research in autoimmunity as defective glycan structures have been described on serum immunoglobulins and defects in glycan biosynthesis have been connected to a congenital autoimmune syndrome and T-cell receptor dysfunction.

Mesenchymal stromal cells (MSC) are a type of multipotent stem cells with immunosuppressive effects. Among other immunosuppressive factors, MSC have been found to secrete Gal-1.

Objectives

Our aim was **to study *in vitro* effect and *in vivo*, systemic immunosuppressive properties of Gal-1 in an immunosuppressive cell type (MSC).** Model of the *in vitro* analysis included co-culturing of activated T-cells as target and MSCs, as Gal-1 producing effector cells. To understand the *in vivo* effect of Gal-1 in MSC's immunosuppressive property streptozotocin induced diabetes mouse model was used. Since Gal-1 is produced by activated T-cells, we wished to **clarify the localization of the lectin (e.g. whether it is secreted or not) and whether extracellular Gal-1-induced apoptosis is affected by the *de novo* expressed Gal-1 in activated T-cells.** Localization of *de novo* Gal-1 in T-cells was ascertained using flow cytometry and fluorescence microscopy and apoptosis studies were carried out on mouse activated T-cells from wild type and Gal-1 knock-out mice.

The role of Gal-1 as an apoptotic factor of effector T-cells makes it a prime candidate for study in autoimmune pathology. Our objective was to **investigate expression**

and function of Gal-1 in the T-lymphocytes of SLE patients by PCR measurements of Gal-1 mRNA levels and apoptosis assays. As the presence of accessible ligands on the cell surface are required for Gal-1 activity, we set out to **study the composition of surface glycans and availability of Gal-1 binding structures on T-cells from SLE, RA and SS patients** using lectin binding assays and analysis of the expression relevant glycosylation enzymes by QPCR.

Materials and Methods

Blood samples from 14 active SLE, 9 inactive SLE, 13 RA and 11 SS patients as well as 16 healthy donors were collected. T-cells were stimulated with 1 $\mu\text{g/ml}$ Phytohaemagglutinin for 72 h at 37 °C.

For apoptosis assays HeLa^{mock} or HeLa^{Gal} cells were plated with activated human T-cells and co-cultured for 16 hours. Co-culture experiments for murine T-cells were carried out in a similar fashion, but with mouse bone marrow MSC as Gal-1 producing cells.

Gal-1 apoptosis steps were measured using flow cytometry and fluorescent microscopy techniques.

Gal-1 mRNA levels as well as levels of glycosylation enzymes were measured with real time qPCR, while fluorescein labelled lectin and Gal-1 binding ability of T-cells was measured using flow cytometry.

Results

The Gal-1 effect is mediated by surface presentation of the lectin on producing cells or extracellular matrix. To understand the contribution of Gal-1 to the immunoregulatory function of MSCs, wild type (wtMSC) and Gal-1 knock-out ($\text{MSC}^{\text{gal-/-}}$) MSCs, isolated from mouse bone marrow were studied in *in vivo* and *in vitro* experiments. In both the STZ-induced diabetes and DTH models, Gal-1 knockout MSC were as effective as wtMSCs in reducing blood glucose level and elevating survival time. These results confirmed that Gal-1 did not participate in the MSCs' immunosuppressive effect when MSCs act systemically. However, mouse wtMSCs triggered apoptosis of activated T-cells in a direct co-culture whereas $\text{MSC}^{\text{gal-/-}}$ did not. The steps of MSC-induced T-cell death included exposure of extracellular phosphatidylserine, ceramide release followed by decreased mitochondrial potential, caspase activation and DNA fragmentation. Apoptosis induction was shown to require direct MSC-T-cell interaction. Physical separation of the cells resulted in the failure of apoptosis induction.

It has been known that T-cells produce Gal-1 upon activation suggesting that it could function as an autocrine apoptotic factor. Earlier we showed that Gal-1 was expressed but not secreted in healthy T-cells, hence excluding an autocrine effect. To clarify the role of *de novo* expressed Gal-1, activated mouse wild type or Gal-1 knock-out T-cells were cultured together with mouse MSCs as Gal-1 source. Gal-1 deficient T-cells displayed significantly reduced response to extracellular Gal-1 compared to wild type, Gal-1 expressing T-cells. Similar results were obtained from Jurkat cells as well, indicating that intracellular Gal-1 regulated the apoptotic response for extracellular Gal-1.

With this in mind, we evaluated Gal-1 expression in activated T-cells of healthy controls, active and treated SLE patients. Active SLE T-cells expressed the lowest level of Gal-1 mRNA compared to healthy or inactive SLE T-cells. One remarkable result was that Gal-1 expression returned to healthy levels after remission.

From our previous results we hypothesized that low expression of Gal-1 in pathological T-cells from SLE

patients could affect their sensitivity to extracellular Gal-1's apoptotic effect. T-cells were co-cultured with deficient (HeLa^{mock}) or Gal-1 producing (HeLa^{Gal}) HeLa cells. Activated T-cells from healthy controls and from SLE patients in remission responded with apoptosis to Gal-1 while T-cells from active SLE patients were unresponsive.

Another reason for this unresponsiveness could be the lack of Gal-1 binding motifs on cell surface glycans, or the inhibition of Gal-1 binding by an altered glycan structure. The cell surface glycosylation of activated autoimmune T-cells from rheumatoid arthritis (RA), Sjögren's syndrome (SS) and SLE patients was analyzed and compared to that of healthy control T-cells using a panel of fluorescently labelled plant lectins. Healthy T-cells, RA and SS patient T-cells could bind similar amounts of Gal-1 before activation, but SLE patient T-cells bound slightly higher amounts compared to controls. The amount of bound Gal-1 was significantly elevated in healthy individuals and in RA patients after activation whereas SLE and SS individuals showed much less pronounced change. All

patient groups could bind significantly less of the lectin than controls after activation. Resting T-cells from SLE patients showed higher ConA, WGA and SNA binding than control T-cells. Upon activation of T-cells, alteration in glycosylation was different in the case of SLE and SS cells. Changes in ConA, LCA, WGA and PHA-L binding to SLE, and LCA and PHA binding to SS T-cells were significantly lower than in controls.

The expression levels of enzyme coding genes involved in glycosylation were examined. Neuraminidase 1 (NEU1), a desialylating enzyme was expressed at significantly lower level in SLE, RA and SS T-cells compared to control T-cells. Since sialyltransferases and neuraminidases catalyse opposite reactions, the gene expression ratios of sialyltransferases and NEU1 were calculated to highlight the sialylation status at expression level in T-cells. Significantly higher ST6GAL1/NEU1 were observed in RA and SS T-cells, and ST3GAL6/NEU1 ratio was elevated in SLE, RA and SS T-cells compared to healthy donor T-cells. It should be

noted that ST6GAL1/NEU1 ratio also correlated with SLEDAI scores of SLE patients.

Summary

- Our findings indicate that Gal-1, although a potent immunosuppressive factor, does not have a role in MSCs **systemic** anti-inflammatory effect *in vivo*.
- Gal-1 expressing MSCs induce T-cell apoptosis in *in vitro* co-culture system on a Gal-1 dependent fashion with identical mechanism as soluble Gal-1 does.
- Endogenous Gal-1 is not secreted from T-cells however it essentially contributes to extracellular Gal-1 induced apoptosis by an unknown mechanism.
- Activated SLE T-cells express lower amounts of Gal-1 mRNA pointing that intracellular Gal-1 can be a relevant factor in escaping extracellular Gal-1 mediated apoptosis.
- Cell surface glycosylation is altered in SLE T-cells including lower complexity of surface glycan chains and elevated sialylation, both changes inhibiting Gal-1 binding to T-cells. Hence defective apoptotic response of SLE may derive from pathological Gal-1 expression and glycosylation. T-cells from SS patients showed similar alterations while RA T-cells showed a shift to sialylation.

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- I. Ákos Hornung^{*}, Magdolna Deák^{*}, Julianna Novák, Dmytro Demidenko, Enikő Szabó, Ágnes Czibula, Roberta Fajka-Boja, Éva Kriston-Pál, Éva Monostori, László Kovács. **Abnormal regulation of T-cell function by galectin-1 in systemic lupus erythematosus.** Controversies in Rheumatology and Immunology conference (Sorrento, Italy 2015)
- II. Ákos Hornung^{*}, Magdolna Deák^{*}, Julianna Novák, Enikő Szabó, Ágnes Czibula, Roberta Fajka-Boja, Éva Kriston-Pál, Éva Monostori, László Kovács. **Novel role for galectin-1 in T-cell apoptosis regulation and its relevance to systemic lupus erythematosus.** European Workshop of Rheumatology Research (Budapest, Hungary 2015)
- III. Ákos Hornung^{*}, Magdolna Deák^{*}, Julianna Novák, Enikő Szabó, Ágnes Czibula, Roberta Fajka-Boja, Éva Kriston-Pál, Éva Monostori, László Kovács. **Insights into the physiology of galectin-1 mediated T-cell apoptosis regulation, and its dysfunction in systemic lupus erythematosus.**

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*Authors contributed equally