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Endogenous galectin-3 expression levels modulate immune responses in galectin-3 transgenic mice

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ABSTRACT

Galectin-3 (Gal-3), a β-galactoside-binding mammalian lectin, is involved in cancer progression and metastasis. However, there is an unmet need to identify the underlying mechanisms of cancer metastasis mediated by endogenous host galectin-3. Galectin-3 is also known to be an important regulator of immune responses. The present study was aimed at analysing how expression of endogenous galectin-3 regulates host immunity and lung metastasis in B16F10 murine melanoma model. Transgenic Gal-3$^{+/-}$ (hemizygous) and Gal-3$^{-/-}$ (null) mice exhibited decreased levels of Natural Killer (NK) cells and lower NK mediated cytotoxicity against YAC-1 tumor targets, compared to Gal-3$^{+/+}$ (wild-type) mice. On stimulation, Gal-3$^{+/-}$ and Gal-3$^{-/-}$ mice splenocytes showed increased T cell proliferation than Gal-3$^{+/+}$ mice. Intracellular calcium flux was found to be lower in activated T cells of Gal-3$^{-/-}$ mice as compared to T cells from Gal-3$^{+/+}$ and Gal-3$^{+/-}$ mice. In Gal-3$^{-/-}$ mice, serum Th1, Th2 and Th17 cytokine levels were found to be lowest, exhibiting dysregulation of pro-inflammatory and anti-inflammatory cytokines balance. Marked decrease in serum IFN-γ levels and splenic IFN-γR1 (IFN-γ Receptor 1) expressing T and NK cell percentages were observed in Gal-3$^{-/-}$ mice. On recombinant IFN-γ treatment of splenocytes *in vitro*, Suppressor of Cytokine Signaling (SOCS) 1 and SOCS3 protein expression was higher in Gal-3$^{-/-}$ mice compared to that in Gal-3$^{+/+}$ and Gal-3$^{+/-}$ mice; suggesting possible attenuation of Signal Transducer and Activator of Transcription (STAT) 1 mediated IFN-γ signaling in Gal-3$^{-/-}$ mice. The ability of B16F10 melanoma cells to form metastatic colonies in the lungs of Gal-3$^{+/+}$ and Gal-3$^{-/-}$ mice remained comparable, whereas it was found to be reduced in Gal-3$^{+/-}$ mice. Our data indicates that complete absence of endogenous host galectin-3 facilitates lung metastasis of B16F10 cells in mice, which may be contributed by dysregulated immune responses resulting from decreased NK cytotoxicity, disturbed serum Th1, Th2, Th17 cytokine milieu, reduced serum IFN-γ levels and attenuation of splenic STAT1 mediated IFN-γ signalling in Gal-3$^{-/-}$ mice.

1. Introduction

Galectin-3 is a unique chimera-type member of the β-galactoside-binding mammalian galectin family. It is a 31 kDa protein composed of an N-terminal domain, a repetitive collagen-like sequence rich in glycine, proline and tyrosine and a C-terminal carbohydrate recognition domain (CRD) (Houzelstein et al., 2004). Galectin-3 can be found within the nucleus, in the cytoplasm, on the cell surface and in the extracellular compartment, depending on the cell type and the proliferative status (Moutsatsos et al., 1987; Perillo et al., 1998; Sato and Hughes, 1994). Thus, it is a ubiquitously expressed molecule with diverse physiological functions based on its subcellular and extracellular localization. Galectin-3 is involved in various biological processes such as maintenance of cellular homeostasis, organogenesis, immune responses, angiogenesis, tumor invasion and metastasis (Califice et al., 2004; Dagher et al., 1995; Dumic et al., 2006; Liu, 2005; Liu et al., 2002; Nakahara et al., 2005; Ochieng et al., 2004; Wang et al., 2004).

Abbreviations: Gal-3, galectin-3; *Lgals3*, lectin galactoside-binding soluble 3; NK, natural killer; Th, T helper; T_{reg}, regulatory T cells; PMA, phorbol 12-myristate 13-acetate; Iono, ionomycin; mAb, monoclonal antibody; FBS, fetal bovine serum; PBS, phosphate buffered saline; FACS, fluorescence-activated cell sorting; CFSE, carboxyfluorescein succinimidyl ester; STAT, signal transducer and activator of transcription; SOCS, suppressor of cytokine signaling.

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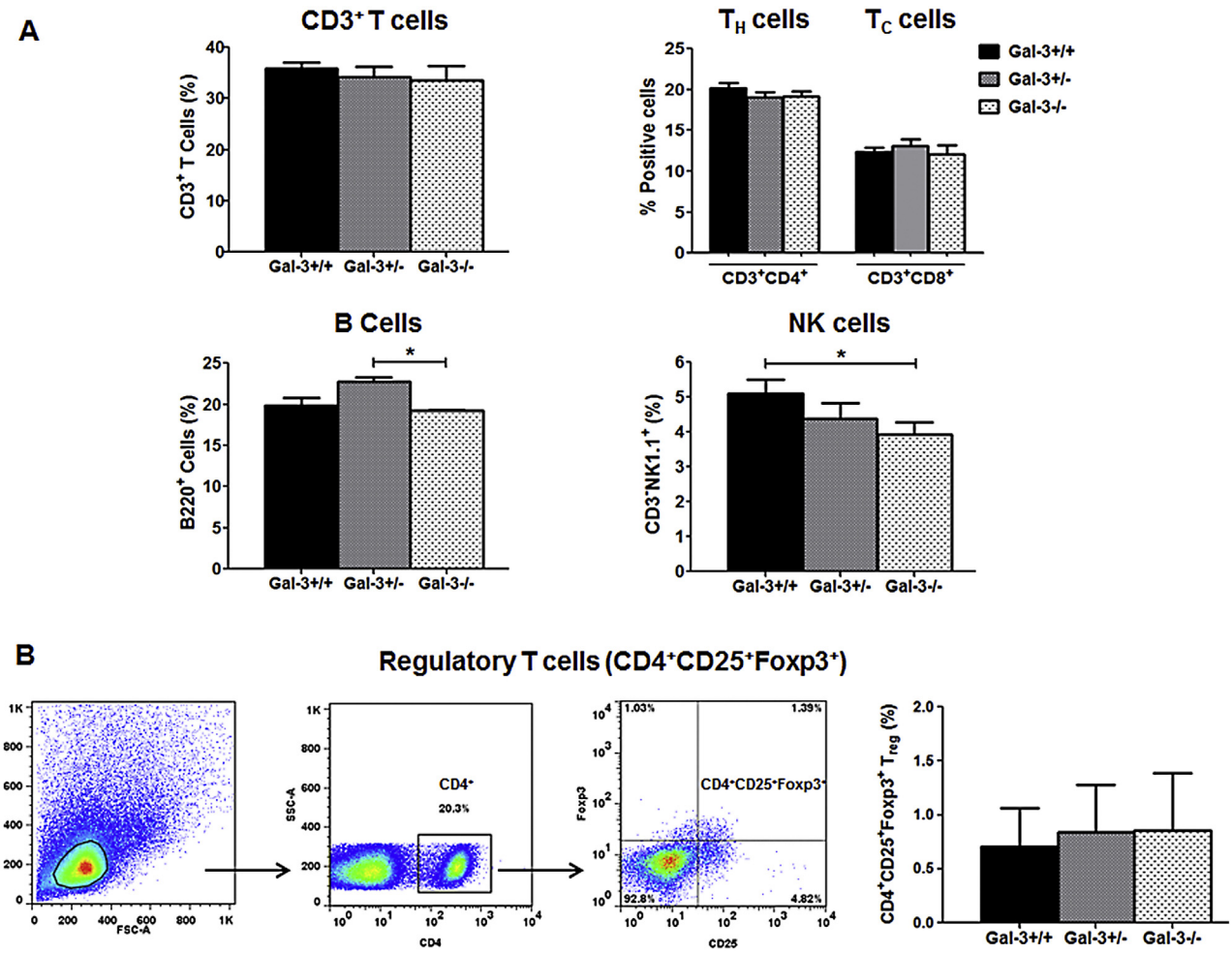

Fig. 1. Immune cell subsets in Gal-3$^{+/+}$, Gal-3$^{+/-}$ and Gal-3$^{-/-}$ mice. Single cell suspensions of spleen cells were prepared from Gal-3$^{+/+}$, Gal-3$^{+/-}$ and Gal-3$^{-/-}$ mice. The cells were fixed, subjected to surface or intracellular antibody staining and analyzed by multicolor flow cytometry. (A) Frequencies of T cells (CD3$^+$), helper T cells (T_H, CD3$^+$CD4$^+$), cytotoxic T cells (T_C, CD3$^+$CD8$^+$), B cells (B220$^+$) and NK cells (CD3$^-$NK1.1$^+$) in mice splenocytes. (B) Gating strategy and percentages of CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells in mice splenocytes. The graphs represent consolidated data of 5 independent experiments. $^*P < 0.05$.

Involvement of galectin-3 in various steps of cancer progression and metastasis has been extensively documented (Fortuna-Costa et al., 2014; Funasaka et al., 2014; Liu and Rabinovich, 2005; Newlaczyl and Yu, 2011; Radosavljevic et al., 2012). Most studies have demonstrated the effect of galectin-3 produced either by the tumor cells themselves or that of the endogenous host galectin-3 on the properties of tumor cells. In the first case depending on the subcellular localization, variety of effects on the tumor cell properties have been demonstrated (Califice et al., 2004; Fortuna-Costa et al., 2014; Liu et al., 2002; Liu and Rabinovich, 2005; Lotz et al., 1993; Nakahara et al., 2005). Using genetic manipulation techniques, effect of ectopic expression or complete knockdown of galectin-3 in cancer cell lines has been assessed through *in vitro* and *in vivo* approaches (Honjo et al., 2001; Yoshii et al., 2001). The effects of host galectin-3 on the tumor cells are exerted via tumor cell surface carbohydrates associated with cancer progression, like TF-antigens on mucinous oligosaccharides (Almogren et al., 2012; Yu, 2007; Yu et al., 2007; Zhao et al., 2010) or poly *N*-acetyl lactosamine substituted *N*-oligosaccharides (Agarwal et al., 2014; Agarwal et al., 2015; Dange et al., 2015; Dange et al., 2014; Srinivasan et al., 2009). However, how the levels of expression of endogenous galectin-3 in the host influence tumor growth and metastasis remains poorly understood till date.

Galectin-3 has been found to be expressed in highest amounts on majority of the tissue compartments of lung and constitutively on lung vascular endothelium in mice (Dange et al., 2014; Krishnan et al., 2005). Previous studies using B16F10 murine melanoma model have shown that, interactions between galectin-3 on the mice lung endothelium and its high affinity ligand poly-*N*-acetyl lactosamine (polyLacNAc) on β1,6 branched *N*-oligosaccharides present on B16F10 melanoma cells facilitates B16F10 colonization in the lungs of the mice (Dange et al., 2014; More et al., 2015; Srinivasan et al., 2009). The studies done in transgenic *Lgals3* mice that are wild type or null for galectin-3 expression have reported contradictory research findings related to primary tumor growth as well as metastatic frequency. A study using C57BL/6 wild-type and galectin-3-null mice has shown that primary subcutaneous B16F10 melanoma tumor growth did not differ between these two groups. However, the number of lung metastatic colonies in wild-type mice was significantly increased in comparison to that observed in galectin-3-null mice (Comodo et al., 2013). Another group has reported that deletion of galectin-3 in the host attenuates lung metastasis of B16F1 malignant melanoma by modulating tumor adhesion and NK cell activity. This study has focused on B16F1 cells, a variant of B16 melanoma possessing lower metastatic potential than B16F10 cells (Radosavljevic et al., 2011). Conversely, it has also been shown that in Gal-3$^{-/-}$ mice, both *Apc* intestinal tumors and *PyMT* mammary gland tumors appear at the same frequency as in Gal-3$^{+/+}$ animals. Further, galectin-3 deletion did not influence the frequency of dissemination of *PyMT* tumors to lungs. Thus, there