

## Psoriasis in humans is associated with downregulation of galectins in dendritic cells

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**ABSTRACT.**

We have investigated the expression and role of galectin-1 and other galectins in psoriasis and in the Th1/Th17 effector and dendritic cell responses associated with this chronic inflammatory skin condition. To determine differences between psoriasis patients and healthy donors, galectins expression was analyzed by RT-PCR assays in skin samples and specifically on epidermal and peripheral blood dendritic cells by immunofluorescence and flow cytometry. In skin of healthy donors galectins 1, 3 and 9 were expressed in a high proportion of Langerhans cells. Also, galectins were differentially expressed in peripheral blood dendritic cell subsets; galectin-1 and galectin-9 were highly expressed in peripheral myeloid dendritic cells compared with plasmacytoid dendritic cells. We found that non-lesional as well as lesional skin samples from psoriasis patients had low levels of galectin-1 at mRNA and protein level in parallel with low levels of IL-10 mRNA compared with skin from healthy patients. However, only lesional skin samples expressed high levels of Th1/Th17 cytokines. The analysis of galectin-1 expression on dendritic cells showed that this protein was downregulated in Langerhans cells as well as in peripheral blood CD11c<sup>+</sup> DCs from psoriasis patients. Addition of galectin-1 to co-cultures of human monocyte-derived dendritic cells with autologous T lymphocytes from psoriasis patients attenuated the Th1 response. Conversely, blockade of galectin binding increased IFN-gamma production and inhibited IL-10 secretion in co-cultures of monocyte-derived dendritic cells with CD4<sup>+</sup> T cells. Our results suggest a model in which galectin-1 downregulation contributes to the exacerbation of the Th1/Th17 effector response in psoriasis patients.

Key words: Psoriasis, galectin, immunoregulation, skin dendritic cells, myeloid dendritic cells, plasmacytoid cells

## INTRODUCTION.

Galectins are a family of highly-conserved glycan-binding proteins. Although initially described as mediators of developmental processes, members of the galectin family are now known to play important roles in the innate and adaptive immune responses [1, 2].

Growing evidence indicates that galectin-1 (gal-1) functions as a negative regulator of the inflammatory response. *In vitro* studies revealed that gal-1 limits the immune response, for example promoting apoptosis of Th-1 cells, inducing IL-10, or down-regulating pro-inflammatory cytokines [3, 4]. Exogenous gal-1 has immunosuppressive and anti-inflammatory effects in experimental models of inflammation and autoimmunity such as inflammatory bowel disease, autoimmune retinal disease, autoimmune diabetes, and collagen-induced arthritis [5-8]. In addition, gal-1-deficient mice show augmented Th1 and Th17 responses and enhanced susceptibility to autoimmune neuroinflammation [3].

Similarly to gal-1, gal-9 down-regulates Th-1 and Th17 responses, and is involved in the suppression induced by CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells through its interaction with the Th-1-specific cell surface molecule TIM-3 [9, 10]. Studies in models of inflammatory disease, such as complex immune-induced arthritis, allergic asthma and diabetes, support an anti-inflammatory role for gal-9 [10-12]. Unlike gal-1, gal-3 can both positively and negatively regulate the inflammatory response, depending on factors such as the specific inflammatory conditions or the type of target cell [13].

Experimental evidence indicates an involvement of Th1 and Th17 responses in the pathogenesis of psoriasis [14], a chronic inflammatory skin

disease affecting 2 to 3% of the human population. Moreover, a recent study described a beneficial effect of gal-9 treatment in a model of IL-23–induced, psoriasis-like skin inflammation [15]. To examine the potential role of galectins in the immunopathogenesis of psoriasis, we analyzed the expression of gal-1, gal-3 and gal-9 in psoriasis patients, and related this to the levels of Th1/Th17 cytokines. We show that expression of gal-1 is decreased both in epidermal dendritic cells (DCs) and in peripheral blood myeloid DCs (PBDCs) from these patients. Functional assays showed that gal-1 inhibits the Th1 response in co-cultures of monocyte-derived DCs (moDCs) from psoriasis patients with autologous peripheral blood lymphocytes.

## **MATERIAL AND METHODS**

### **Study subjects and sample collection**

The study was approved by the institutional review board and the independent ethics committee of the Hospital Universitario de la Princesa, and conformed to the Declaration of Helsinki principles. After giving informed consent, 10 healthy individuals and 24 untreated patients with moderate to severe psoriasis were enrolled. Patients recruited to the study had a psoriasis area and severity index (PASI)  $\geq 10$  or a body surface area (BSA)  $\geq 10\%$ . The following washout periods were established: 14 days for topical corticosteroids, 28 days for conventional systemic treatment including corticosteroids, methotrexate, cyclosporin, acitretin and phototherapy, and 84 days for biologic anti-TNF agents. Skin punch biopsies (10-mm) were obtained from lesional plaque-type psoriatic skin and non-lesional skin. Biopsies from non-lesional skin were at least 5 cm from a lesional plaque, and were taken preferentially from non sun-exposed areas. In some cases 20 ml of venous peripheral blood were obtained. Normal skin and peripheral blood samples were obtained from 10 surgical patients without cutaneous disease. Data regarding gender, age, duration of psoriasis and clinical disease severity (PASI and BSA) are presented in Table 1.

### **RT-PCR analysis of cytokines and lectins**

Total RNA was isolated using TRIzol reagent (Invitrogen). One microgram of RNA was used to generate cDNA. IL-17a, IL-21, IL-23, IL-12b, IL-10, IL-27, gal-1, gal-3, gal-4, gal-8, gal-9, dectin-1, DCIR and CLEC5A were amplified using the Power SYBR green PCR master mix (Applied Biosystems, Warrington UK).

mRNA was normalized to GAPDH levels. Primer sequences are shown in Table 2.

### **Double immunofluorescence staining and measurement of skin gal-1 expression**

Skin biopsies were embedded in OCT and frozen. After fixation and permeabilization, skin sections were blocked with 100 ug/ml human gamma-globulin (Sigma-Aldrich, St Louis MO) and 5% FCS in PBS. Sections were incubated with anti-gal-1, anti-gal-3 anti-gal7, or anti-gal-9, all at 10 µg/ml for 1 h and incubated with alexa Fluor 488-DAG. Mouse anti-human CD1a (T6), or anti-CD11c. Biotin-coupled mouse anti-human MHC-class II (DCIS 1/21) and rat anti-human Alexa-fluor 647-Langerin (Dendritics, Lyon, France) were also used. Sections were examined with a Leica DMR immunofluorescence microscopy under the same acquisition conditions. Images were analyzed with ImageJ (<http://imagej.softonic.com>) to quantify gal-1 expression. For the analysis of gal-1 expression, fluorescence intensity was determined in regions of interest (ROIs) drawn on CD1a+, Langerin+ or CD11c+, MHC class-II+ cells

Detailed description of antibodies and other reagents is in supplemental data.

### **Coculture of moDCs with autologous PBLs.**

MoDCs from psoriasis patients preloaded with SEE (0.1 µg/ml) were cocultured with autologous PBLs (1:10) in the presence or absence of h gal-1 (2 µM) for 5 days. Cells were harvested and intracellular levels of IFN-gamma were analyzed in T cells using flow cytometry.

**Flow cytometry and cell sorting.**

To analyze galectin surface expression on primary circulating DCs, PBMCs were stained with the following mouse anti-human monoclonal antibodies: PerCP-HLA-DR, FITC-CD3, FITC-CD14, FITC-CD16, FITC-CD19, FITC-CD20, PE-CD123 and V450-CD11c, and goat polyclonal anti-gal-1, anti-gal-3 or anti-gal-9 followed by Alexa fluor 647-DAG. Myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were isolated from PBMCs by cell sorting in a FACSAria cell sorter. DCs were lysed and galectin expression determined by western blot.

Epidermal and dermal single-cell suspensions from healthy skin donors were obtained after separation of the epidermis and dermis as described [16]. Skin cell suspensions were stained with the following antibodies: FITC-HLA-DR and PE-anti-CD1a, and anti-gal-1, anti-gal-3 or anti-gal-9 followed by Alexa fluor 647-DAG for epidermal cells and Pacific blue-CD11c, Pacific orange-CD45, FITC-HLA-DR for dermal cells. Dead cells were excluded using 7 ADD.

**Statistical analysis.**

Data were analyzed with GraphPad Prism (GraphPad Software Inc, San Diego, USA). The Kruskal-Wallis test and Mann-Whitney U-test were used when appropriate. Spearman test was used for correlation analysis. Differences were considered significant at  $p < 0.05$ .



## RESULTS.

### Galectin expression is defective in psoriatic skin

To explore the potential role of galectins in the immunopathogenesis of psoriasis, we conducted an RT-PCR analysis of the expression of gal-1, gal-3, gal-4, gal-8 and gal-9 and the lectins DCIR, CLEC5A and dectin-1 in non-lesional and lesional skin samples from 24 psoriasis patients and control samples from 10 healthy subjects. Compared with the skin of healthy subjects, lesional and non-lesional skin biopsies from psoriasis patients showed low expression of gal-1 mRNA (Fig. 1A,  $p < 0.05$ ). Levels of gal-3 and gal-4 were also low in lesional skin, but expression in non-lesional (asymptomatic) samples was similar to the level in skin from healthy patients. In contrast, gal-8 and gal-9 expression was similar in all three types of sample (Fig 1A). Dectin-1 was detected at high level in lesional skin, in agreement with a recent report [17]. Conversely, we did not observe differences in the expression of CLEC5A and DCIR.

Increasing evidence suggests that effector Th1 and Th17 cells are key players in the pathogenesis of psoriasis [18]. In order to validate our skin samples, we analyze the mRNA expression of Th1 and Th17 cytokines. RT-PCR analysis revealed high expression of IL-17a, IL-21, IL-12b, IL-23 of in lesional skin of psoriasis patients compared with both non-lesional skin and skin samples from healthy donors ( $p < 0.05$  Fig. 1B and supplemental Fig. 1). We found higher IL-27 mRNA expression in lesional skin of psoriasis patients than in skin samples from healthy donors (Supplemental Fig. 1), according to previous data [19]. IL-27 expression was also above in asymptomatic skin from psoriasis patients, but this difference was not statistically significant. In contrast, expression of the anti-

inflammatory cytokine IL-10 was low in lesional and non-lesional skin from psoriasis patients compared with healthy donor skin ( $p < 0.05$ , Fig. 1B).

Statistical analysis showed that low levels of gal-1 are associated with high levels of IL-17 ( $p < 0.0001$ ,  $r^2 = 0,463$ ), and with low levels of IL-10 ( $p < 0.0001$ ,  $r^2 = 0,393$ ) (Fig. 1C).

### **Gal-1, gal-3 and gal-9 are expressed in Langerhans cells.**

Dendritic cells are central participants in immune mechanisms, and abnormal activation and function of DCs are associated with several chronic inflammatory conditions, including psoriasis [20]. Despite the importance of galectins in immune response regulation, there has been little study of their role in human primary DCs. To study the immune regulatory function of gal-1, gal-3 and gal-9 in psoriasis, we examined their expression on skin DCs. Immunofluorescence analysis of MHC class-II<sup>+</sup> cells in skin sections from healthy subjects revealed expression of gal-1 and gal-9 in MHC class-II<sup>+</sup> stellate cells within the epidermis of healthy individuals, pointing to expression on Langerhans cells (LCs) (Fig. 2A). Gal-3 and gal-7, a galectin characteristic of keratinocytes [21], were highly expressed on epidermal keratinocytes (Fig. 2A). The high expression of gal-3 epidermal cells masks the possible expression of these galectins on MHC-class II epidermal cells. In addition we analyze the expression of gal-1, gal-3 and gal-9 on LC isolated from epidermal sheets. Flow cytometry analysis clearly showed the expression of gal-1, gal-3 and gal-9 on LCs (CD1a<sup>+</sup> cells gated for HLA-DR expression, Fig. 2B).

### **Gal-1 expression is reduced in Langerhans cells from psoriasis patients**

Double-immunofluorescence staining of skin sections from psoriasis patients showed weak staining of gal-1 on epidermal CD1a+ cells in comparison with healthy donors. To quantify this difference, we measured the mean fluorescence intensity of gal-1 staining on CD1a+ epidermal cells in skin sections from eight psoriasis patients and eight healthy subjects. (Supplemental Fig. 2A). This analysis showed that levels of gal-1 are lower in epidermal CD1a+ DCs from lesional and non-lesional skin from psoriasis patients compared with healthy donor skin ( $p < 0.05$ ) (Supplemental Fig. 2B). Recently, it has been described that a subpopulation of inflammatory dendritic epidermal cells (IDECs) are CD1a+ [16]. In order to assess the expression of gal-1 specifically in LC, three-color immunostaining was performed in skin samples (Fig. 3A). The analysis of gal-1 expression showed that Langerhans cells (langerin+ CD1a+) from psoriasis patients express low levels of Gal-1 compared with healthy donors (Fig. 3B). The low levels of gal-1 were also corroborated in total lysates of skin biopsies from 2 patients and 2 healthy donors by western blot (Fig. 3C).

### **Gal-1 expression in other populations of skin DCs.**

Due to the lower expression of gal-1 in CD1a+ cells, we analyzed whether there was a differential expression between IDEC CD1a+ and Langerhans cells. The analysis of gal-1 expression in four-color immunostaining (MHC class-II, CD1a, langerin and gal-1) from lesional psoriatic skin revealed that IDECs express very low levels of gal-1 (Fig 4A).

Beside LC, dermal DCs (dDCs) are the other major population of skin DC in healthy skin [22]. An additional population of inflammatory dermal DCs

(CD11c+) has been recently described in patients with psoriasis [23]. The analysis of galectin expression on dDCs by flow cytometry of DCs derived from healthy donor skin showed a mild expression of gal-1 and gal-9 (Fig. 4B and data not shown). Three-color immunostaining (MHC class-II, CD11c and gal-1) of skin samples from psoriasis patients and healthy donors corroborated the low expression of gal-1 in dDCs in healthy donors (Fig. 4C) and showed an even lower expression in psoriasis patients (Supplemental Fig. 3)

### **Galectins are differentially expressed in peripheral DC subsets, and gal-1 expression is defective in peripheral DCs of psoriasis patients**

Like skin, peripheral blood contains distinct DC populations. The main PBDC subsets are myeloid DCs (mDCs, CD11c+) and plasmacytoid DCs (pDCs, CD123+) [24]. These subsets differ in their origin, surface markers, and functions [25]. FACS analysis of galectin expression in PBDCs from healthy donors revealed higher surface expression of gal-1, gal-3 and gal-9 on the surface of mDCs compared with pDCs (Fig. 5A). In contrast, the immunoregulatory molecules ICOS and ICOSL showed no significant differences in expression. Higher expression of gal-1 and gal-9 in mDCs was also detected by western blot of mDCs and pDCs isolated from peripheral blood by cell sorting. We next determined whether galectin expression on peripheral blood mDCs was affected in psoriasis. Flow cytometry analysis revealed significantly lower gal-1 expression on mDCs from psoriasis patients (Fig. 5B). Correlation analysis between disease activity (PASI) and gal-1 expression in mDCs showed that higher disease activity indexes are associated with lower levels of gal-1 (Fig. 5C;  $r^2=-0.5662$ ,  $p=0.03$ ). Expression of gal-3 and gal-9 was

also slightly lower, but these differences were not statistically significant. Levels of ICOS and ICOSL were similar in psoriasis patients and healthy subjects. To determine whether the reduced expression of gal-1 was also observed in other inflammatory skin condition, gal-1 was analyzed in peripheral mDCs from patients with atopic dermatitis. Our results showed that expression of gal-1 in mDCs from atopic patients is similar to the expression observed in healthy subjects (Fig 5D).

### **Inhibition of galectin binding increases IFN-gamma production and decreases IL-10 production by CD4+ T cells**

Gal-1 and gal-9 have been described as negative regulators of the Th1 immune response [3][26]. To assess this, we measured IFN-gamma and IL-10 production in co-cultures of CD4+ T cells with human moDCs preloaded with superantigen staphylococcal enterotoxin E (SEE) in the presence or absence of lactose (50mM), an inhibitor of galectin binding. Intracellular staining revealed that lactose-mediated inhibition of galectin binding augments IFN-gamma production by CD4+ T cells (Fig. 6A). Conversely, lactose inhibited IL-10 production induced by SEE (Fig. 6A).

We next probed the ability of gal-1 to regulate IFN-gamma production in cells from psoriasis patients. moDCs from psoriasis patients were preloaded with SEE and co-cultured autologous PBLs. This analysis showed that exogenously added gal-1 inhibited IFN-gamma production by T cells from psoriasis patients (Fig. 6B). Western blot of total lysates of moDCs from psoriasis patients and healthy donors untreated and treated with LPS showed the lower expression of this protein in cells from psoriasis samples (Fig. 6C).

## DISCUSSION

In this study we show that Langerhans cells and peripheral mDCs from psoriasis patients express low levels of gal-1. In addition, gal-1 is able to reduce the IFN-gamma secretion produced by lymphocytes from these patients. Current understanding of the exacerbated immune response observed in psoriasis and other inflammatory diseases is based mainly on the study of the upregulation of pro-inflammatory molecules. Accordingly, several components of the pathways involved in T-cell activation are well-established targets in psoriasis antibody-based therapies, for example anti-TNF-alpha, anti-LFA-1, anti-LFA-3, and more recently anti-IL-12/IL-23 [14, 27]. However, recent studies acknowledge the importance of anti-inflammatory signals that counterbalance the inflammatory response. Galectins are implicated in the negative regulation of the immune response, participating in processes such as immune cell proliferation [28], apoptosis [29], cellular adhesion and migration [30-32], and modulation of the interactions between T cells and APCs [33].

This study shows that primary human skin DCs express gal-1, gal-3 and gal-9 on their cell surface. Moreover, we detected differential expression of gal-1 and gal-9 in peripheral blood myeloid and plasmacytoid DCs. Although additional studies would be necessary, we could speculate that the differential expression of gal-1 and gal-9 might account for different functions in these cells.

The role of endogenous gal-1 in DCs has recently been studied in a mouse model of encephalomyelitis. Galectin-1, either exogenously supplied or regulated endogenously, drove the differentiation of DCs toward a regulatory function, promoting T cell tolerance [34][15]. Most evidence pointing to gal-1 as a negative regulator of the immune response has been gathered in animal

models of inflammatory diseases, and studies in human disease are comparatively scarce. Here, we demonstrate that psoriasis patients have low expression of gal-1 in peripheral blood myeloid DCs and in LCs of lesional and non-lesional skin that together with previous studies in animal models [34] indicate an important role of gal-1 in the immunopathogenesis of psoriasis. The positive role of gal-1 in IL-10 production has been proposed as one of the mechanisms involved in the inhibition of Th1 and Th17 responses [34-36]. Our results are consistent with this notion and show that blockade of galectin binding reduces the levels of IL-10 during antigen presentation. Together with the lower expression of IL-10 observed in psoriatic skin, these results indicate that defects in gal-1 expression by DCs may promote the Th1 and Th17 immune response in psoriasis, likely by controlling IL-10 expression.

The co-culture experiments showed that galectin inhibition increases IFN-gamma production by CD4<sup>+</sup> T cells in response to antigen presentation by DCs, suggesting that galectins also regulate the production of pro-inflammatory cytokines. Interestingly, IFN-gamma production was decreased by addition of recombinant gal-1 to autologous co-cultures of PBLs with moDCs from psoriasis patients. The effects of gal-1 on T cells are likely due to the binding of cell-surface glycoproteins on these cells. A number of T cell glycoproteins, including CD2, CD3, CD7, CD45 and CD43 have been shown to act as receptors for gal-1 binding and to be involved in gal-1-mediated T cell death [37]. However, the absence of apoptosis induction under the doses used in our assays suggests a non-apoptotic mechanism and a gal-1 partner on activated T cells not described yet. It is known that gal-1 is able to modulate the TNF-alpha and IFN-gamma secretion without affect cell viability [38]. The role of galectins in the

pathogenesis of psoriasis may also be due to their effect on other cell types. In this regard, gal-1 inhibited keratinocyte migration in a model of wound healing [39].

Dendritic cells are important for the induction not only of T-cell immunity, but also of tolerance. The maintenance of peripheral tolerance has been proposed to involve LC migration to the draining lymph node during steady state. However, DC tolerogenicity is not specific to a DC subset or restricted to the immature APC state [40]. The means by which DCs convey tolerance are not entirely clear, but involve the secretion of suppressive cytokines such as IL-10 and the induction of regulatory lymphocytes. The expression of specific galectin members on LCs and myeloid peripheral blood DCs may play an important role in the physiology of these cells.

Our results are consistent with a model in which galectin-1 expression and engagement limit skin inflammatory responses, and suggest that galectin-1 downregulation in human DCs may contribute to the exacerbation of the inflammatory response observed in psoriasis. The increased recognition of galectins as immunoregulatory molecules could lead to new therapeutic approaches to restoring the immune equilibrium lost in psoriasis.



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Abbreviations: dendritic cells (DCs), Langerhans cells (LCs), myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), monocyte-derived dendritic cells (moDCs), superantigen staphylococcal enterotoxin E (SEE), galectin (gal), peripheral blood dendritic cells (PBDCs), antigen presenting cell (APC), Psoriasis area and severity index (PASI).

Statement authors contributions.

HFF conceived and carried out experiments, generation of figures, literature search and writing the manuscript

SPG Patients recruitment, carried out experiments, literature search

PB carried out experiments, generation of figures, literature search

ACA carried out experiments, generation of figures

DNP carried out experiments

SSC carried out experiments

MF conceived experiments

ED Patients recruitment, writing the manuscript

AGD Study design, data interpretation

FSM Study design, data interpretation

List of online supplementary information:

Supplemental Fig. 1

Supplemental Fig. 2

Supplemental Fig. 3

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**Table 1.** *Clinical characteristics of psoriasis patients and healthy subjects.*

	<b>Psoriasis patients</b>	<b>Healthy subjects</b>
<b>SUBJECTS (n)</b>	24	10
<b>SEX (M/F)</b>	13/11	4/6
<b>AGE (years)</b>	49.7 (26-81)	49.1 (44-69)
<b>PASI</b>	20.76 (9.3-53)	
<b>BSA (%)</b>	23 (10-90)	
<b>TIME OF EVOLUTION (y)</b>	22.5 (8-63)	

*Results are expressed as median (interquartile range). M: male; F: female; PASI: Psoriasis Area and Severity Index; BSA: Body Surface Area.*

**Table 2.** Sequence of primers used for RT-PCR

<i>Gen</i>	<i>Forward</i>	<i>Reverse</i>
GADPH	5'AGCCACATCGCTCAGACAC3'	5'GCCCAATACGACCAAATCC3'
<i>GAL-1</i>	5'AGCGGGAGGCTGTCTTTC3'	5'CCTGGTCTGAAGGTGATGC3'
<i>GAL-3</i>	5'GAGCCTACCCTGCCACTG3'	5'AGGCAAAGGCAGGTTATAAGG3'
<i>GAL-4</i>	5'TTTGATCTGTCCATTCGCTG3'	5'CCTGGATTTCGAATGTGTCC3'
<i>GAL-8</i>	5'AGCTTAGGCTGCCATTCG3'	5'CCTGCTAGTAGGTCAACATTAAAGC3'
<i>GAL-9</i>	5'CACACARGCCTTCCAGA3'	5'AAGAGGATCCCGTTCACCAT3'
<i>IL-10</i>	5'TGGGGGAGAACCTGAAGAC3'	5'CCTTGCTCTTGTTTTCACAGG3'
<i>IL-27</i>	5'GCCAGGAGTGAACCTGTACC3'	5'TGGTGGAGATGAAGCAGAGA3'
<i>IL-17A</i>	5'TGGGAAGACCTCATTGGTGT3'	5'GGATTTCTGTTGGGATTGTGAT3'
<i>IL-12B</i>	5'CCCTGACATTCTGCGTTCA3'	5'AGGTCTTGTCCGTGAAGACTCTA3'
<i>IL-21</i>	5'AGGAAACCACCTTCCACAAA3'	5'GAATCACATGAAGGGCATGTT3'
<i>IL-23A</i>	5'TGTTCCCATATCCAGTGTG3'	5'TCCTTTGCAAGCAGAACTGA3'
<i>DECTIN-1</i>	5'CTTTCTCGGCCCCAGACT3'	5'TTGGGTAGCTGTGGTTCTGA3'
<i>DCIR</i>	5'GAGAAGGACTGTGCTAGAATGGA3'	5'TGCAGATTCTGGAAGATGAAATC3'
<i>CLEC5A</i>	5'GGCGTTGGATCAACAACCTCT3'	5'GATCCTGCGGTAGCTGATG3'

## FIGURE LEGENDS.

**Fig. 1. Lesional and non-lesional skin from psoriasis patients expresses low levels of gal-1 and IL-10.** RT-PCR analysis of the indicated lectins (**A**) and cytokines (**B**) genes in non-lesional and lesional skin samples from psoriasis patients and control samples from healthy subjects. Expression levels were normalized to GAPDH. Bars represent means  $\pm$  SEM from 24 lesional (black), 24 non-lesional (grey) and 10 healthy samples (empty). Differences between groups were analyzed by the Kruskal-Wallis and Bonferroni test (\*  $p < 0.05$  compared with healthy). **C.** Correlation analysis of gal-1, IL-17 and IL-10 mRNA levels in psoriasis patients. Correlation was tested using Spearman test. Data correspond to lesional skin in the case of IL-17 correlation and both lesional and non-lesional skin for IL-10.

**Fig. 2 Gal-1, gal-3 and gal-9 are expressed in Langerhans cells in normal skin.** **A.** Double immunofluorescence analysis of skin from a healthy subject for expression of galectins 1, 3, 7 or 9 (green) and MHC-II (red), nuclei were counterstained with Hoechst (blue). Arrowheads mark galectin expression on LCs. **B.** Three-color flow cytometry analysis of galectin expression in DCs isolated ex vivo from the epidermal sheet of normal skin. Cells were gated for HLA-DR expression, and galectin expression was then determined on CD1a+ cells.

**Fig. 3. Langerhans cells from psoriasis patients express low levels of gal-1.** **A.** Immunofluorescence analysis of gal-1 expression in skin sections from a healthy donor and a psoriasis patient. Triple immunofluorescence of CD1a

(red), langerin (magenta) and gal-1 (green) is shown. Arrowheads mark gal-1 expression on LCs. **B.** Quantification of gal-1 expression on Langerhans cells. Gal-1 signal intensity was measured on CD1a<sup>+</sup> langerin<sup>+</sup> cells using ImageJ software. Data are means $\pm$ SD of fluorescence intensity from four psoriasis patients and four healthy controls; at least 50 cells were analyzed for each skin section. **C.** Gal-1 expression in total lysates of skin biopsies from 2 psoriasis patients (one of them from lesional and non-lesional skin) and 2 healthy donors. Vimentin expression was used as loading control.

**Fig 4.** Gal-1 expression in IDECs and dermal DCs (dDC). **A.** Four-color immunofluorescence of lesional skin from psoriasis patients. Skin samples were immunostained with anti-MHC class-II (red), anti-CD1a (blue), anti-langerin (magenta) and anti-gal-1 (green). Gal-1 signal intensity was measured as in Fig. 3 on MHC class II<sup>+</sup> CD1a<sup>+</sup> langerin<sup>+</sup> cells (LCs) and MHC class-II<sup>+</sup> CD1a<sup>+</sup> langerin<sup>-</sup> (IDECs). LCs are indicated by solid line and IDECs by dashed line regions. **B.** Flow cytometry analysis of gal-1 expression in dDCs isolated ex vivo from the dermal sheet of normal skin. Data inside histogram correspond to mean fluorescence intensity (MFI). Three-color immunofluorescence from a healthy donor skin biopsy stained with anti-gal-1 (green), anti-MHC class-II (red) and anti-CD11c (white) is shown. Regions indicate dDCs.

**Fig. 5. Peripheral blood myeloid DCs from psoriasis patients express low levels of gal-1.** **A.** Gal-1, gal-3 and gal-9 are highly expressed in CD11c<sup>+</sup> DCs compared with CD123<sup>+</sup> DCs. PBMCs were obtained from 10 ml samples of peripheral blood from healthy donors, and expression of the indicated



molecules was evaluated by flow cytometry. Data are means $\pm$ SEM from 14 independent experiments. Gal-1 and gal-9 in total lysates from mDCs and pDCs isolated from peripheral blood by cell sorting. **B.** Expression of regulatory molecules in mDCs from psoriasis patients and healthy subjects. Protein expression was determined in CD11c+DCs by flow cytometry as in A. Bars represent means $\pm$ SEM from 12 psoriasis patients and 14 healthy subjects. **C.** Gal-1 expression in mDCs from healthy subjects (n=14), psoriasis patients (n=12), and atopic dermatitis patients (n=6). Differences were analyzed by the Mann-Whitney test, \* p<0.05. **D.** Negative correlation of gal-1 expression in mDCs and disease activity (PASI) in psoriasis patients. Correlation between gal-1 expression and PASI was analysed by Spearman test.

**Fig. 6. Gal-1 inhibits IFN-gamma production in psoriasis patients. A.** Galectin inhibition increases IFN-gamma expression and decreases IL-10 production. SEE-preloaded moDCs and allogeneic CD4<sup>+</sup> T cells both from healthy donors, were co-cultured for 5 days in the presence or absence of lactose (50 mM), and intracellular levels of IFN-gamma (n=5) and production of IL-10 (n=10) were determined by flow cytometry and ELISA, respectively. Differences between treatments were analyzed by paired *t* test. **B.** Gal-1 partially inhibits IFN-gamma production by LT from psoriasis patients. SEE-preloaded moDCs and autologous LT from psoriasis patients were co-cultured for 5 days with or without 2  $\mu$ M gal-1, and intracellular levels of IFN-gamma were determined in CD4<sup>+</sup> T cells by flow cytometry. Results from five psoriasis patients are shown. Differences between treatments were analyzed by the Kruskal Wallis test. **C.** Gal-1 expression in total lysates of moDCs from 1

psoriasis patient and 1 healthy donor is shown. moDCs at 7d with or without LPS were lysed and loaded on a 12% polyacrylamide gel.

**Supplemental Fig. 1. Cytokine expression in skin biopsies from psoriasis patients and healthy donors by RT-PCR.** mRNA expression of Th1 and Th17 cytokines in lesional and non-lesional skin samples from psoriasis patients and samples from healthy subjects. Expression was normalized to GAPDH. Bars represent means $\pm$ SEM. from 24 lesional, 24 non-lesional and 10 healthy samples. Differences between groups were analyzed by the Kruskal-Wallis and Bonferroni Test (\*  $p < 0.05$ ).

**Supplemental Fig. 2. Epidermal CD1a<sup>+</sup> cells from psoriasis patients express low levels of gal-1. A.** Immunofluorescence analysis of gal-1 expression in skin sections from a healthy donor and a psoriasis patient. Double immunofluorescence of CD1a (red) and gal-1 (green) is shown. Arrowheads mark gal-1 expression on CD1a<sup>+</sup> cells. **B.** Quantification of gal-1 expression on CD1a<sup>+</sup> cells. Gal-1 signal intensity was measured on CD1a<sup>+</sup> cells using ImageJ software. Data are means $\pm$ SD of fluorescence intensity from four psoriasis patients and four healthy controls; at least 50 cells were analyzed for each skin section.

**Supplemental Fig. 3. Quantification of gal-1 expression on dDCs.** Gal-1 signal intensity was measured on CD11c<sup>+</sup> MHC class-II<sup>+</sup> cells using ImageJ software in skin samples from lesions and non-lesions of psoriasis patients and healthy subjects. Data are means $\pm$ SD of fluorescence intensity from four psoriasis patients and four healthy controls.

















