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Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10

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Despite their central function in orchestrating immunity, dendritic cells (DCs) can respond to inhibitory signals by becoming tolerogenic. Here we show that galectin-1, an endogenous glycan-binding protein, can endow DCs with tolerogenic potential. After exposure to galectin-1, DCs acquired an interleukin 27 (IL-27)-dependent regulatory function, promoted IL-10-mediated T cell tolerance and suppressed autoimmune neuroinflammation. Consistent with its regulatory function, galectin-1 had its highest expression on DCs exposed to tolerogenic stimuli and was most abundant from the peak through the resolution of autoimmune pathology. DCs lacking galectin-1 had greater immunogenic potential and an impaired ability to halt inflammatory disease. Our findings identify a tolerogenic circuit linking galectin-1 signaling, IL-27-producing DCs and IL-10-secreting T cells, which has broad therapeutic implications in immunopathology.

For a long time, attention has been focused on the capacity of dendritic cells (DCs) to elicit T cell responses[1]; however, these cells can also induce peripheral tolerance by promoting the differentiation of regulatory T cells (T_{reg} cells), including $CD4^+CD25^+Foxp3^+$ T_{reg} cells and type 1 T_{reg} cells[2–4]. Several stimuli may influence the 'decision' of DCs to become tolerogenic, including recognition of apoptotic cells[5], interaction with stromal cells[6,7] and exposure to soluble factors such as interleukin 10 (IL-10; A001243)[8], vasoactive intestinal peptide (VIP)[9] and 1,25-dihydroxyvitamin D_3 (ref. 10). In addition, DCs modified by $CD4^+CD25^+Foxp3^+$ T_{reg} cells may become tolerogenic and drive the differentiation of IL-10-producing type 1 T_{reg} cells[11], which suggests a link among distinct regulatory cell populations; this effect requires the secretion of IL-27, an IL-12-related cytokine with paradoxical pro- and anti-inflammatory effects[11–14].

During the past few years, there has been increasing appreciation of the effect of protein-glycan interactions on the regulation of innate and adaptive immunity[15]. Galectin-1, an evolutionarily conserved glycan-binding protein, elicits a broad spectrum of biological functions mainly by suppressing T cell responses[16]. Blockade of galectin-1 expression results in heightened T cell–mediated tumor rejection and more secretion of T helper type 1 (T_H1) cytokines[17,18]. Moreover, galectin-1-deficient ($Lgals1^{-/-}$) mice show augmented T_H1 and T_H-17 responses and are considerably more susceptible to immune-mediated fetal rejection and autoimmune disease than are their wild-type counterparts[19,20].

Given the notable plasticity of DCs, induction of a tolerogenic profile might be exploited to attenuate autoimmune diseases and prevent graft rejection[4]. In contrast, silencing DC regulatory pathways might augment vaccination efficiency and potentiate tumor immunotherapeutic strategies[21]. Here we show that galectin-1, either exogenously supplied or endogenously regulated, drove the differentiation of DCs with a regulatory function; these DCs promoted T cell tolerance, blunted T_H-17 and T_H1 responses and suppressed autoimmune inflammation through mechanisms involving IL-27 and IL-10.

RESULTS

Galectin-1 alters human DC function

In a search for potential mechanisms responsible for the broad anti-inflammatory activity of galectin-1 (ref. 16), we conducted an integrated study of the effect of this protein on human and mouse DCs. We first compared monocyte-derived immature DCs differentiated for 7 d with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 in the absence (iDC) or presence (iDC_{Gal1}) of galectin-1. Recombinant galectin-1 bound to human monocytes in a dose- and carbohydrate-dependent way (**Supplementary Fig. 1a**) and interfered with the differentiation of immature DCs, as reflected by the lower expression of CD1a and the higher expression of CD14 in iDC_{Gal1} compared with that of control iDC (**Fig. 1a**). Furthermore, iDC_{Gal1} had a lower capacity to endocytose fluorescein isothiocyanate

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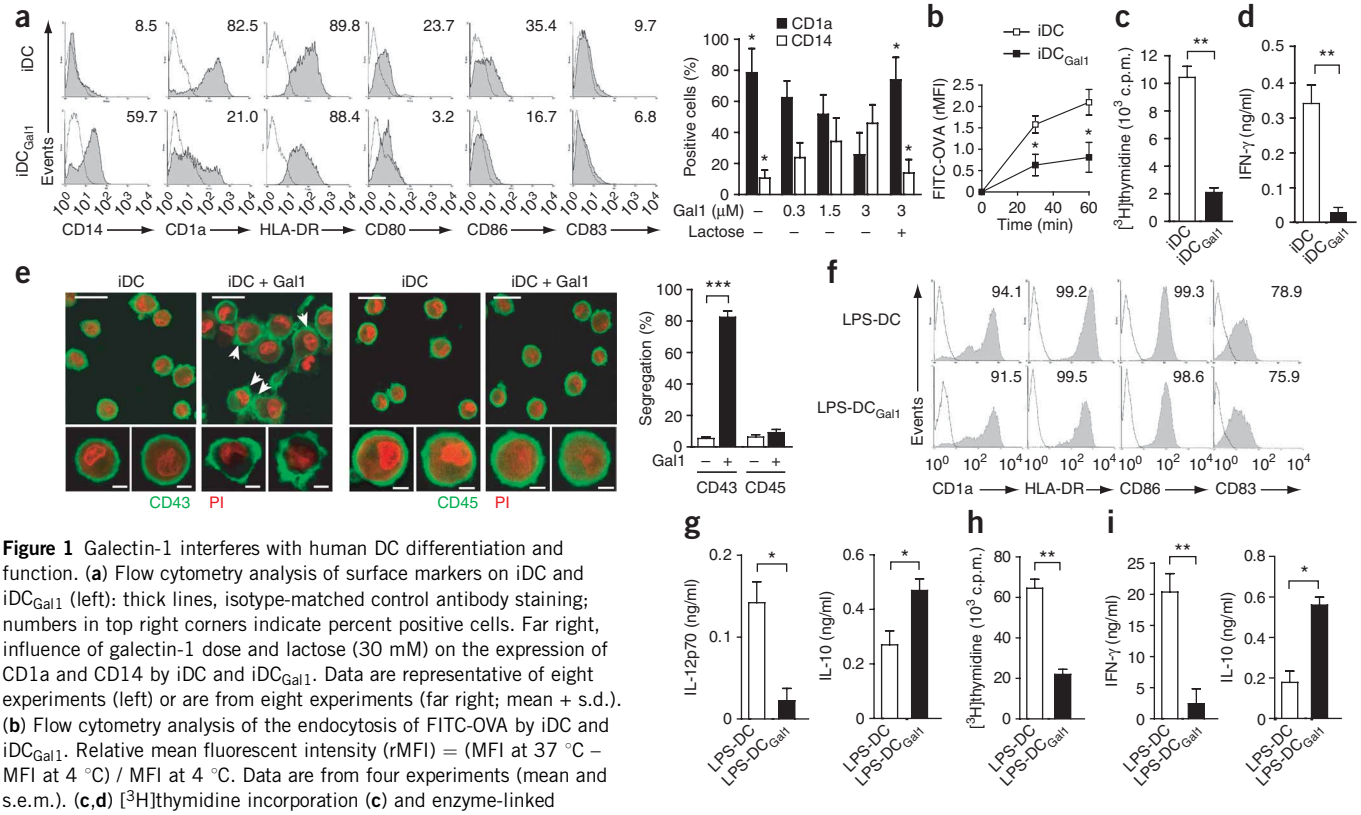

Figure 1 Galectin-1 interferes with human DC differentiation and function. (**a**) Flow cytometry analysis of surface markers on iDC and iDC$_{Gal1}$ (left): thick lines, isotype-matched control antibody staining; numbers in top right corners indicate percent positive cells. Far right, influence of galectin-1 dose and lactose (30 mM) on the expression of CD1a and CD14 by iDC and iDC$_{Gal1}$. Data are representative of eight experiments (left) or are from eight experiments (far right; mean + s.d.). (**b**) Flow cytometry analysis of the endocytosis of FITC-OVA by iDC and iDC$_{Gal1}$. Relative mean fluorescent intensity (rMFI) = (MFI at 37 °C – MFI at 4 °C) / MFI at 4 °C. Data are from four experiments (mean and s.e.m.). (**c**,**d**) [^{3}H]thymidine incorporation (**c**) and enzyme-linked immunosorbent assay (ELISA) of IFN-γ production (**d**) by allogeneic CD4$^+$ T cells cultured for 5 d with iDC or iDC$_{Gal1}$ (DC/T cell ratio, 1:5). Data are from five experiments (mean + s.e.m.). (**e**) Laser confocal microscopy (left) of iDCs incubated with galectin-1 or buffer and stained with antibody to human CD43 (anti–human CD43) or anti–human CD45 and propidium iodide (PI). Scale bars, 20 μm (top row) or 5 μm (bottom row). Right, frequency of cells showing segregation of CD43 or CD45 to membrane patches (five randomly selected fields with at least 50 cells per condition). Data are from three experiments (mean + s.e.m.). (**f**) Flow cytometry analysis of surface markers on DCs matured for 24 h with LPS alone (LPS-DC) or with LPS and galectin-1 (LPS-DC$_{Gal1}$). Thick lines, isotype-matched control antibody staining. Numbers in top right corners indicate percent positive cells. Data are representative of six experiments. (**g**) ELISA of cytokines in supernatants of LPS-DC$_{Gal1}$ or LPS-DC. Data are from four experiments (mean + s.e.m.). (**h**,**i**) [^{3}H]thymidine incorporation (**h**) and ELISA of cytokine production (**i**) by allogeneic CD4$^+$ T cells cultured for 5 d with LPS-DC$_{Gal1}$ or LPS-DC. Data are from four experiments (mean + s.e.m.). Gal1 is used at a concentration of 3 μM unless stated otherwise. $*P < 0.05$; $**P < 0.01$. $***P < 0.001$ (Student's *t*-test).

(FITC)-labeled ovalbumin (OVA) than that of control iDC (**Fig. 1b**). To examine the ability of galectin-1-differentiated DCs to prime T cells, we cultured iDC$_{Gal1}$ or control iDC together with alloreactive CD4$^+$ T cells. Priming with iDC$_{Gal1}$ resulted in poor T cell proliferation and negligible production of interferon-γ (IFN-γ) compared with that of control iDC (**Fig. 1c,d**). Thus, galectin-1 impairs the differentiation and allostimulatory capacity of human monocyte-derived iDC. As predicted by their 'permissive' glycophenotype[22], iDC also bound galectin-1 in a carbohydrate-dependent way (**Supplementary Fig. 1b**), and the glycoreceptor CD43, but not CD45, segregated to membrane patches in iDC exposed to galectin-1 (**Fig. 1e**).

Although DCs stimulated with lipopolysaccharide (LPS) showed similar upregulation of maturation markers in the presence or absence of galectin-1 (**Fig. 1f**), galectin-1-exposed LPS-stimulated DCs (LPS-DC$_{Gal1}$) produced less IL-12p70 and more IL-10 than did DCs matured with LPS alone (LPS-DC; **Fig. 1g**). Furthermore, allogeneic CD4$^+$ T cells primed with LPS-DC$_{Gal1}$ showed weak proliferation and synthesized less IFN-γ and more IL-10 than did CD4$^+$ T cells primed with control LPS-treated DCs (**Fig. 1h,i**). However, we found no difference in the amount of transforming growth factor-β1 (TGF-β1) or IL-4 in supernatants of these cultures (data not shown). Thus, regardless of a mature cell surface phenotype, exposure to galectin-1 overrides the ability of LPS to induce IL-12-producing, fully competent DCs.

Galectin-1 imparts a regulatory program in human DCs

To determine whether DCs matured in a galectin-1-enriched microenvironment had enhanced immunoregulatory potential, we tested the capacity of these cells to inhibit mixed leukocyte reactions (MLRs) stimulated by fully competent, LPS-matured DCs. Galectin-1-conditioned DCs (LPS-DC$_{Gal1}$) suppressed proliferation and IFN-γ production and enhanced IL-10 secretion induced by fully competent DCs (LPS-DC) in a dose-dependent way (**Fig. 2a–c**). To understand the mechanistic basis of this effect, we first examined the ability of galectin-1 to control activation of the transcription factor STAT3, as it critically regulates the tolerogenic function of DCs[21]. Maturation of DCs with LPS in the presence of galectin-1 resulted in more STAT3 phosphorylation than did exposure of DCs to LPS alone (**Fig. 2d**). Furthermore, addition of the Jak2 kinase–STAT3 inhibitor AG490 during DC maturation substantially diminished the regulatory capacity of LPS-DC$_{Gal1}$ (**Fig. 2e**). In contrast, exposure to galectin-1 during DC maturation did not affect degradation of the transcription factor NF-κB inhibitor IκBα or NF-κB DNA-binding activity (**Supplementary Fig. 2a,b**). Moreover, we found no difference in the frequency of apoptotic cells in DCs cultured in the absence or presence of galectin-1 during differentiation or maturation (**Supplementary Fig. 3a–d**).

To determine whether allogeneic T cells were rendered regulatory after exposure to LPS-DC$_{Gal1}$, we purified CD4$^+$ T cells from MLR cultures and analyzed their suppressive capacity. Although we detected

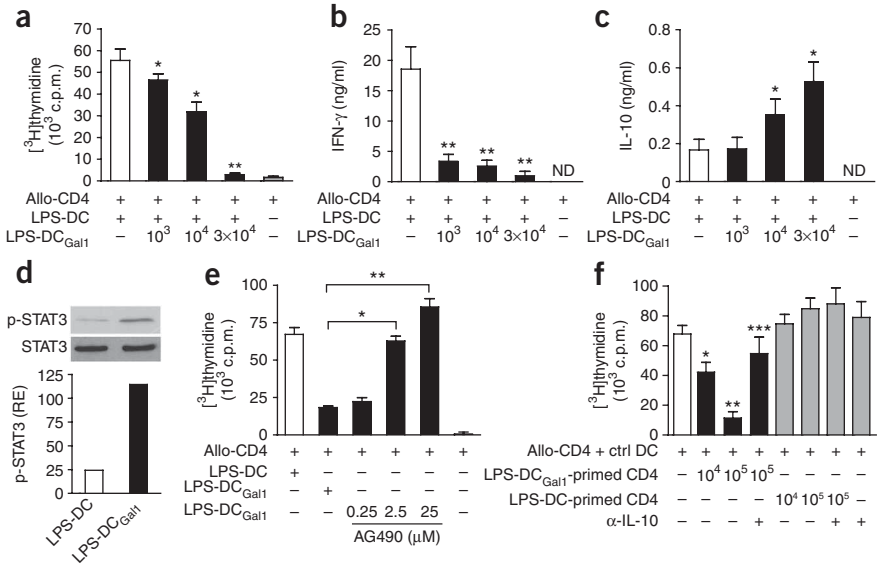

Figure 2 Galectin-1 imparts a regulatory program in human mature DCs. (**a**) [^{3}H]thymidine incorporation by human allogeneic CD4$^+$ T cells cultured for 5 d with LPS-DC$_{Gal1}$ or LPS-DC. (**b**,**c**) ELISA of IFN-γ (**b**) and IL-10 (**c**) in supernatants of the CD4$^+$ T cells described in **a**. ND, not detected. $^*P < 0.05$ and $^{**}P < 0.01$, versus control LPS-DC (Student's *t*-test; **a**–**c**). Data are from four experiments (**a**–**c**; mean + s.e.m.). (**d**) Immunoblot analysis of phosphorylated STAT3 (p-STAT3) in LPS-DC$_{Gal1}$ or LPS-DC; below, band intensity relative to that of STAT3 (RE, relative expression). Data are representative of three experiments. (**e**) [^{3}H]thymidine incorporation by human allogeneic CD4$^+$ T cells (Allo-CD4) cultured for 5 d with LPS-DC or LPS-DC$_{Gal1}$ previously exposed to various doses of the Jak2-STAT3 inhibitor AG490 during maturation. $^*P < 0.01$; $^{**}P < 0.001$ (Student's *t*-test). Data are from three experiments (mean + s.e.m.). (**f**) Inhibition of allogeneic T cell responses by human CD4$^+$ T cells exposed for 5 d to allogeneic LPS-DC$_{Gal1}$ (LPS-DC$_{Gal1}$-primed CD4) or LPS-DC (LPS-DC-primed CD4), then purified by cell sorting and added to a secondary MLR culture (Allo-CD4 + ctrl DC), evaluated as [^{3}H]thymidine incorporation in the presence or absence of IL-10-neutralizing antibody (α-IL-10) or irrelevant isotype-matched control (data not shown). $^*P < 0.05$ and $^{**}P < 0.01$, versus untreated secondary MLR (Allo-CD4 + ctrl DC); $^{***}P < 0.05$, versus human CD4$^+$ T cells primed with LPS-DC$_{Gal1}$ (1×10^5) added to secondary MLR (Student's *t*-test). Data are from three experiments (mean + s.e.m.).

no variation in the frequency of CD4$^+$CD25$^+$Foxp3$^+$ T$_{reg}$ cells (**Supplementary Fig. 4**), CD4$^+$ T cells primed with LPS-DC$_{Gal1}$ but not those primed with control LPS-DC suppressed T cell proliferation in a dose-dependent way (**Fig. 2f**). This effect was prevented by an IL-10-specific neutralizing antibody (**Fig. 2f**), which suggested that LPS-DC$_{Gal1}$ promoted the differentiation of IL-10-secreting anti-inflammatory T cells. Thus, in the presence of inflammatory stimuli, galectin-1 drives the generation of human DCs with a mature cell surface phenotype but greatly enhanced regulatory potential.

Galectin-1 induces IL-27-producing mouse tolerogenic DCs

Given the regulatory effects of galectin-1 on human DCs, we analyzed the effect of this lectin on the differentiation of mouse DCs to further evaluate their tolerogenic potential *in vivo*. Culture of bone marrow cells in the presence of GM-CSF and galectin-1 (BM-DC$_{Gal1}$) led to the differentiation of cells with lower expression of CD11c and higher expression of CD45RB (a marker associated with regulatory DCs[7,8]) than that of bone marrow cells differentiated with GM-CSF alone (BM-DC; **Fig. 3a**). This effect involved protein-glycan interactions, as it was prevented by the disaccharide lactose (**Fig. 3a**). After exposure to LPS, BM-DC$_{Gal1}$ adopted a cell surface phenotype similar to that of fully mature BM-DCs (data not shown).

To examine the functional profile of BM-DC$_{Gal1}$, we analyzed cytokine production and allostimulatory capacity. We detected concomitant upregulation of the p28 and EBI3 subunits of IL-27 on BM-DC$_{Gal1}$ relative to their expression on control BM-DC (**Fig. 3b**), which was sustained after maturation of these cells (**Supplementary Fig. 5**). In addition, we detected more IL-6 and IL-10 and less IL-12p70 in BM-DC$_{Gal1}$ than in control BM-DC after maturation with LPS (**Fig. 3c**). In agreement with that, priming of alloreactive CD4$^+$ T cells with CD11c^{lo}CD45RB$^+$ BM-DC$_{Gal1}$ resulted in less proliferation, less synthesis of IFN-γ and IL-17 and more production of IL-10 than that of T cells primed with control BM-DC (**Fig. 3d**,**e**). Notably, blockade of IL-27 with an IL-27p28-specific antibody completely eliminated this immunoregulatory effect (**Fig. 3d**,**e**). However, neutralization of TGF-β did not reverse suppression of allogeneic T cell responses mediated by BM-DC$_{Gal1}$, whereas blockade of the IL-10 receptor produced a partial effect (**Fig. 3d**). Again, we found no difference in the frequency of CD4$^+$CD25$^+$Foxp3$^+$ T$_{reg}$ cells (**Supplementary Fig. 6**) or in the amount of IL-4 or TGF-β in allogeneic CD4$^+$ T cells primed either with BM-DC$_{Gal1}$ or control BM-DC (data not shown).

The IL-27 receptor, which consists of the gp130 common receptor chain associated with the IL-27 receptor α-subunit (IL-27Rα (A002911); also called WSX-1), mediates the induction of IL-10-producing T cells triggered by IL-27 (refs. 11–14). To evaluate the contribution of this pathway to the tolerogenic circuit initiated by galectin-1, we analyzed the ability of BM-DC$_{Gal1}$ to prime naive CD4$^+$ T cells purified from the spleens of IL-27Rα-deficient (*Il27ra*$^{-/-}$) mice. CD4$^+$ T cells lacking IL-27Rα were refractory to the regulatory effects of BM-DC$_{Gal1}$ (**Fig. 3f**,**g**). Similarly, BM-DC$_{Gal1}$ were unable to suppress proliferation or modulate cytokine production in CD4$^+$ T cells isolated from IL-10-deficient (*Il10*$^{-/-}$) mice (**Fig. 3f**,**g**), thus substantiating the contribution of DC-derived IL-27 and T cell–derived IL-10 to the immunoregulatory effects of galectin-1. Similar to the human system, incorporation of the Jak2-STAT3 inhibitor AG490 during mouse DC maturation partially abrogated the regulatory function of BM-DC$_{Gal1}$ (**Fig. 3h**), consistent with the ability of galectin-1 to trigger STAT3 phosphorylation (**Fig. 3i**). Collectively, these data underscore the involvement of galectin-1–saccharide interactions in driving the differentiation of CD11c^{lo}CD45RB$^+$ tolerogenic DCs, which favor the induction of IL-10-producing T cells through IL-27- and STAT3-dependent mechanisms.

DC$_{Gal1}$ promote T cell tolerance *in vivo*

To determine whether DCs differentiated in a galectin-1-enriched microenvironment have enhanced regulatory function *in vivo*, we first pulsed BM-DC$_{Gal1}$ and control BM-DC with OVA (producing OVA-DC$_{Gal1}$ and OVA-DC, respectively) and transferred these cells into syngeneic naive mice. Then, 7 d later, we injected mice with OVA in complete Freund's adjuvant (CFA). We then analyzed antigen-specific proliferation and cytokine production in splenocytes of mice given

OVA-DC$_{Gal1}$ or control OVA-DC after *ex vivo* restimulation with OVA. We found vigorous antigen-specific proliferation, large amounts of IFN-γ and IL-17 and small amounts of IL-10 in splenocytes of mice given control OVA-DC before antigenic challenge (**Fig. 4a**,**b**). In contrast, adoptive transfer of OVA-DC$_{Gal1}$ resulted in weak antigen-specific proliferation, less secretion of IFN-γ and IL-17 and more synthesis of IL-10 in splenocytes of recipient mice (**Fig. 4a**,**b**). Coinjection of OVA-DC$_{Gal1}$ and control OVA-DC resulted in less antigen-specific proliferation, less secretion of IFN-γ and IL-17 and more IL-10 production by *ex vivo*–restimulated splenocytes than that of cells from mice that received only control OVA-DCs (**Fig. 4a**,**b**), suggestive of a dominant tolerogenic effect of antigen-pulsed DC$_{Gal1}$, which prevented the immunostimulatory ability of antigen-pulsed control DCs. To examine the contributions of IL-27 and IL-10 pathways to this tolerogenic effect, we injected OVA-DC$_{Gal1}$ or control OVA-DC into *Il27ra*$^{-/-}$ or *Il10*$^{-/-}$ mice. Disruption of either the IL-27 or IL-10 pathway mostly abrogated the tolerogenic effect of OVA-DC$_{Gal1}$ (**Fig. 4a**,**b**). We found no change when we injected unpulsed DCs or unpulsed DC$_{Gal1}$ into wild-type mice (**Fig. 4a**,**b**), *Il27ra*$^{-/-}$ mice or *Il10*$^{-/-}$ mice (data not shown).

Tumor lysate–pulsed DCs can elicit effective antitumor responses and protect mice against challenge with viable tumor cells[23]. However, the protective function of DCs could be thwarted by a tolerogenic microenvironment[21]. To investigate the regulatory function of DC$_{Gal1}$ in an *in vivo* setting of pathophysiologic relevance, we pulsed BM-DC$_{Gal1}$ and control BM-DC with lysates of B16 melanoma cells (producing Lys-DC$_{Gal1}$ and Lys-DC, respectively) and did tumor protection assays. We immunized mice twice at 7-day intervals with tumor-pulsed or unpulsed BM-DC$_{Gal1}$ or control BM-DC, challenged mice 14 d later with viable B16 cells and monitored tumor progression[17]. Mice immunized with tumor-pulsed BM-DC showed 80% or more inhibition of tumor growth compared with the growth of tumors from mice pretreated with vehicle control or unpulsed BM-DC (**Fig. 4c**). However, we found no substantial inhibition of tumor growth

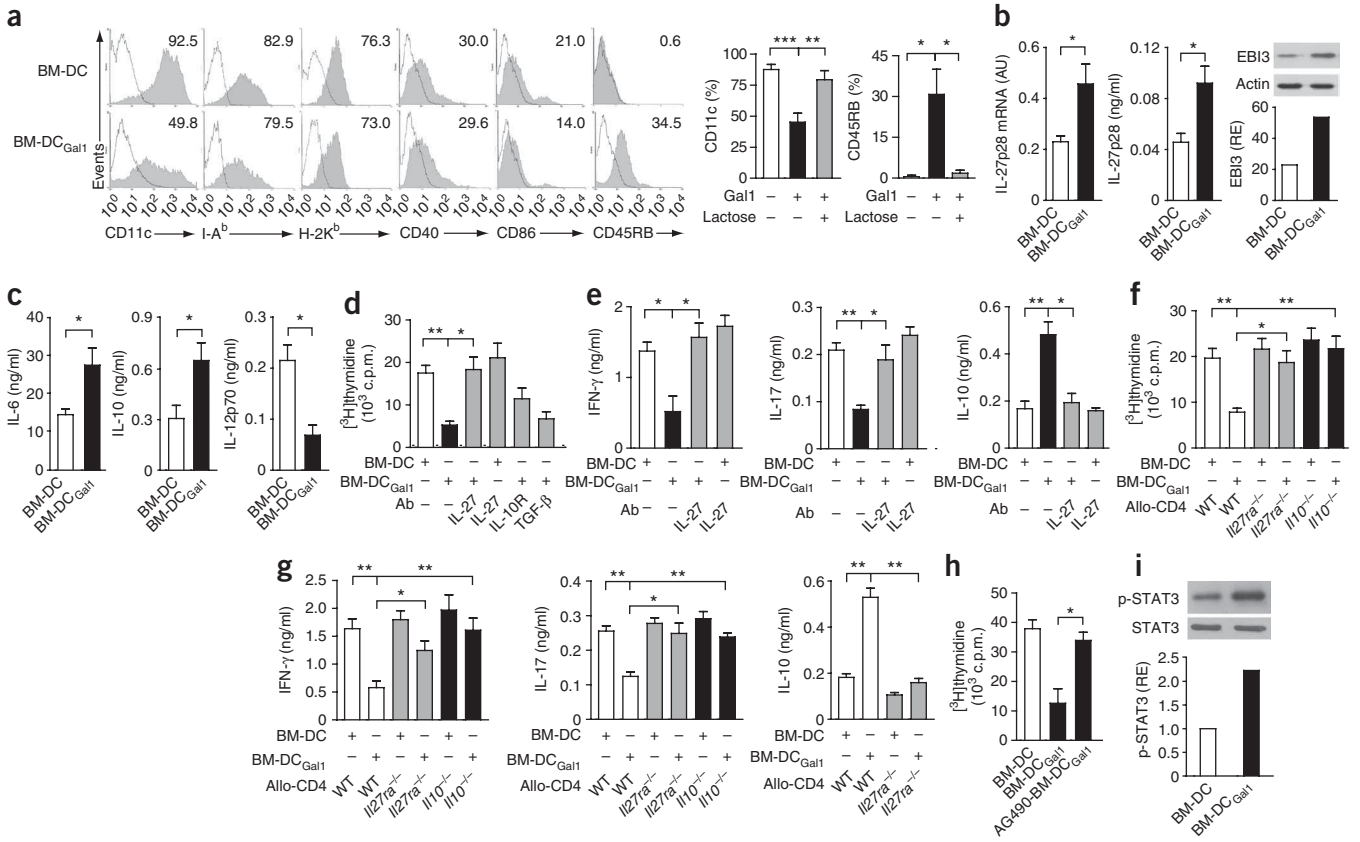

Figure 3 Galectin-1 endows mouse DCs with IL-27-dependent tolerogenic potential. (**a**) Flow cytometry analysis (left) of surface markers on bone marrow–derived DCs differentiated with recombinant mouse GM-CSF in the absence (BM-DC) or presence (BM-DC$_{Gal1}$) of galectin-1 (3 μM). Thick lines, isotype-matched control antibody staining. Numbers in plots indicate percent positive cells. Right, lactose-dependent modulation (30 mM) of CD11c and CD45RB expression. Data are representative of nine experiments (left) or are from nine experiments (right; mean + s.d.). (**b**) Real-time quantitative RT-PCR analysis (left) of the expression of IL-27p28 mRNA by the cells described in **a**; results are presented in arbitrary units (AU) relative to GAPDH mRNA (encoding glyceraldehyde phosphate dehydrogenase). Middle, ELISA of IL-27p28 in supernatants of the cells described in **a**; right, immunoblot analysis of IL-27EBI3 (below, band intensity relative to that of actin). Data are representative of three experiments. (**c**) ELISA of cytokines in supernatants of BM-DC or BM-DC$_{Gal1}$ stimulated with LPS. Data are from four experiments (mean + s.e.m.). (**d**,**e**) [^{3}H]thymidine incorporation (**d**) and cytokine production (**e**) by BALB/c CD4$^+$ splenocytes stimulated for 5 d with C57BL/6 BM-DC or BM-DC$_{Gal1}$ (DC/T cell ratio, 1:10) in the presence or absence of neutralizing antibodies (Ab) or control immunoglobulin G (data not shown). Data are from three experiments (mean + s.e.m.). (**f**,**g**) [^{3}H]thymidine incorporation (**f**) and cytokine production (**g**) by C57BL/6 wild-type (WT), *Il27ra*$^{-/-}$ or *Il10*$^{-/-}$ CD4$^+$ splenocytes stimulated for 5 d with BALB/c BM-DC or BM-DC$_{Gal1}$ (DC/T cell ratio, 1:10). Data are from three experiments (mean + s.e.m.). (**h**) [^{3}H]thymidine incorporation by BALB/c CD4$^+$ splenocytes cultured for 5 d with C57BL/6 control BM-DC or BM-DC$_{Gal1}$ exposed to LPS in the absence (BM-DC$_{Gal1}$) or presence (AG490-BM-DC$_{Gal1}$) of 2.5 μM AG490. Data are from three experiments (mean + s.e.m.). (**i**) Immunoblot analysis of phosphorylated STAT3 on BM-DC or BM-DC$_{Gal1}$; below, band intensity relative to that of STAT3. Data are representative of three experiments. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (Student's *t*-test).

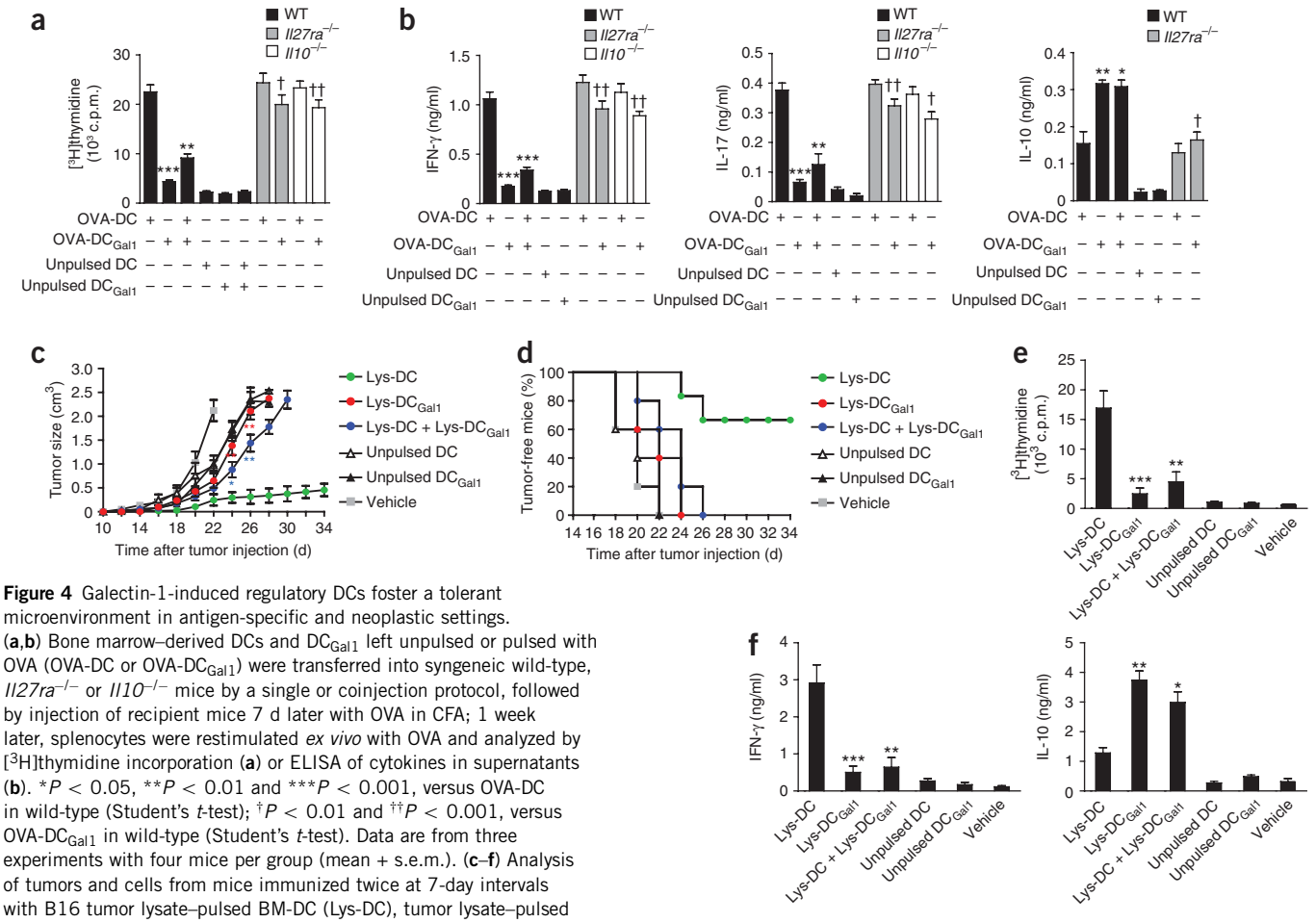

Figure 4 Galectin-1-induced regulatory DCs foster a tolerant microenvironment in antigen-specific and neoplastic settings. (**a**,**b**) Bone marrow–derived DCs and DC_{Gal1} left unpulsed or pulsed with OVA (OVA-DC or OVA-DC_{Gal1}) were transferred into syngeneic wild-type, *Il27ra*$^{-/-}$ or *Il10*$^{-/-}$ mice by a single or coinjection protocol, followed by injection of recipient mice 7 d later with OVA in CFA; 1 week later, splenocytes were restimulated *ex vivo* with OVA and analyzed by [^{3}H]thymidine incorporation (**a**) or ELISA of cytokines in supernatants (**b**). $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, versus OVA-DC in wild-type (Student's *t*-test); $^{\dagger}P < 0.01$ and $^{\dagger\dagger}P < 0.001$, versus OVA-$DC_{Gal1}$ in wild-type (Student's *t*-test). Data are from three experiments with four mice per group (mean + s.e.m.). (**c**–**f**) Analysis of tumors and cells from mice immunized twice at 7-day intervals with B16 tumor lysate–pulsed BM-DC (Lys-DC), tumor lysate–pulsed BM-DC_{Gal1} (Lys-DC_{Gal1}) or their unpulsed counterparts or vehicle control, by single or coinjection protocol, and further challenged with viable B16 melanoma cells. (**c**) Kinetics of tumor growth. $^*P < 0.05$ and $^{**}P < 0.01$, versus Lys-DC (Student's *t*-test). (**d**) Kaplan-Meier analysis of tumors. $^*P < 0.05$, Lys-DC versus Lys-DC_{Gal1} or a combination of Lys-DC and Lys-DC_{Gal1}. (**e**,**f**) [^{3}H]thymidine incorporation (**e**) and ELISA of cytokines in supernatants (**f**) of tumor-draining lymph node cells obtained 2 weeks after tumor challenge and restimulated for 72 h with irradiated B16 cells. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, versus Lys-DC (Student's *t*-test). Data are from three experiments with five to six mice per group (mean ± s.e.m. in **c**; mean + s.e.m. in **e**,**f**).

when we immunized mice with tumor-pulsed BM-DC_{Gal1} (**Fig. 4c**). When challenged with viable melanoma cells, all mice immunized with tumor-pulsed BM-DC_{Gal1} developed progressively enlarging tumors at a rate similar to that of mice that received unpulsed BM-DC or vehicle control, which led to uniform terminal morbidity by about 20–25 d after challenge (**Fig. 4d**). In contrast, 60% of mice immunized with fully competent, tumor-pulsed control BM-DC remained tumor-free for about 30 d after inoculation (**Fig. 4d**). Coinjection of tumor-pulsed BM-DC and tumor-pulsed BM-DC_{Gal1} resulted in accelerated tumor growth compared with that of mice that received only tumor-pulsed BM-DC (**Fig. 4c**,**d**), confirming a dominant tolerogenic effect of tumor-pulsed BM-DC_{Gal1}, which prevented the protective effect of tumor-pulsed control BM-DCs. Consistent with that, lymph node cells from mice that received tumor-pulsed BM-DC_{Gal1} or a mixture of tumor-pulsed BM-DCs and tumor-pulsed BM-DC_{Gal1} showed poor proliferative responses, less synthesis of IFN-γ and enhanced secretion of IL-10 compared with that of lymph node cells from mice immunized with fully competent, tumor-pulsed BM-DCs (**Fig. 4e**,**f**). Thus, DCs differentiated in a galectin-1-enriched microenvironment cannot elicit an effective T cell response against tumor challenge and instead skew the cytokine balance to foster a tolerant milieu at sites of tumor growth.

DC_{Gal1} halt autoimmune inflammation through IL-27 and IL-10

To evaluate whether DC_{Gal1} might be used as a therapeutic tool to halt autoimmune diseases, we examined the tolerogenic effects of these cells in experimental autoimmune encephalomyelitis (EAE), a T cell–mediated disorder widely used as a model of multiple sclerosis[24,25]. We immunized mice with the encephalitogenic peptide (amino acids 35–55) of myelin oligodendrocyte glycoprotein (MOG(35–55)) and monitored disease progression. We injected mice that reached a clinical score of 1 (disease onset) with syngeneic MOG(35–55)-pulsed BM-DC (MOG-DC) or BM-DC_{Gal1} (MOG-DC_{Gal1}). Treatment with MOG-DC_{Gal1} resulted in much less clinical severity than that of mice treated with control MOG-DC (**Fig. 5a** and **Supplementary Table 1a**). In addition, areas of inflammation and demyelination were less pronounced in spinal cord sections from mice treated with peptide-pulsed MOG-DC_{Gal1} than in those from mice injected with peptide-pulsed control MOG-DCs (**Fig. 5b**). Similar numbers of MOG-DC and MOG-DC_{Gal1} reached draining lymph nodes of treated mice (**Supplementary Fig. 7**), which suggested a lack of effect of galectin-1 on the *in vivo* migratory pattern of DCs.

T_H-17 and T_H1 effector cells provide distinct but essential contributions to autoimmune neuroinflammation[24,25]. Lymph node cells from mice treated with MOG-DC_{Gal1} showed much less antigen-specific

proliferation, much less MOG(35–55)-specific production of IL-17 and IFN-γ and much more IL-10 than did lymph node cells from mice treated with peptide-pulsed control DCs (**Fig. 5c,d**). To examine the contribution of the IL-27 pathway to the therapeutic effect of DC_{Gal1}, we injected syngeneic MOG-DC_{Gal1} into *Il27ra*$^{-/-}$ or wild-type mice at the day of EAE onset. Disruption of the IL-27 pathway mostly abrogated the tolerogenic effect of MOG-DC_{Gal1}, which were not able to limit disease severity or modulating cytokine secretion (**Fig. 5e,f** and **Supplementary Table 1b**). Furthermore, MOG-DC_{Gal1} also lacked therapeutic efficacy when injected at the day of disease onset

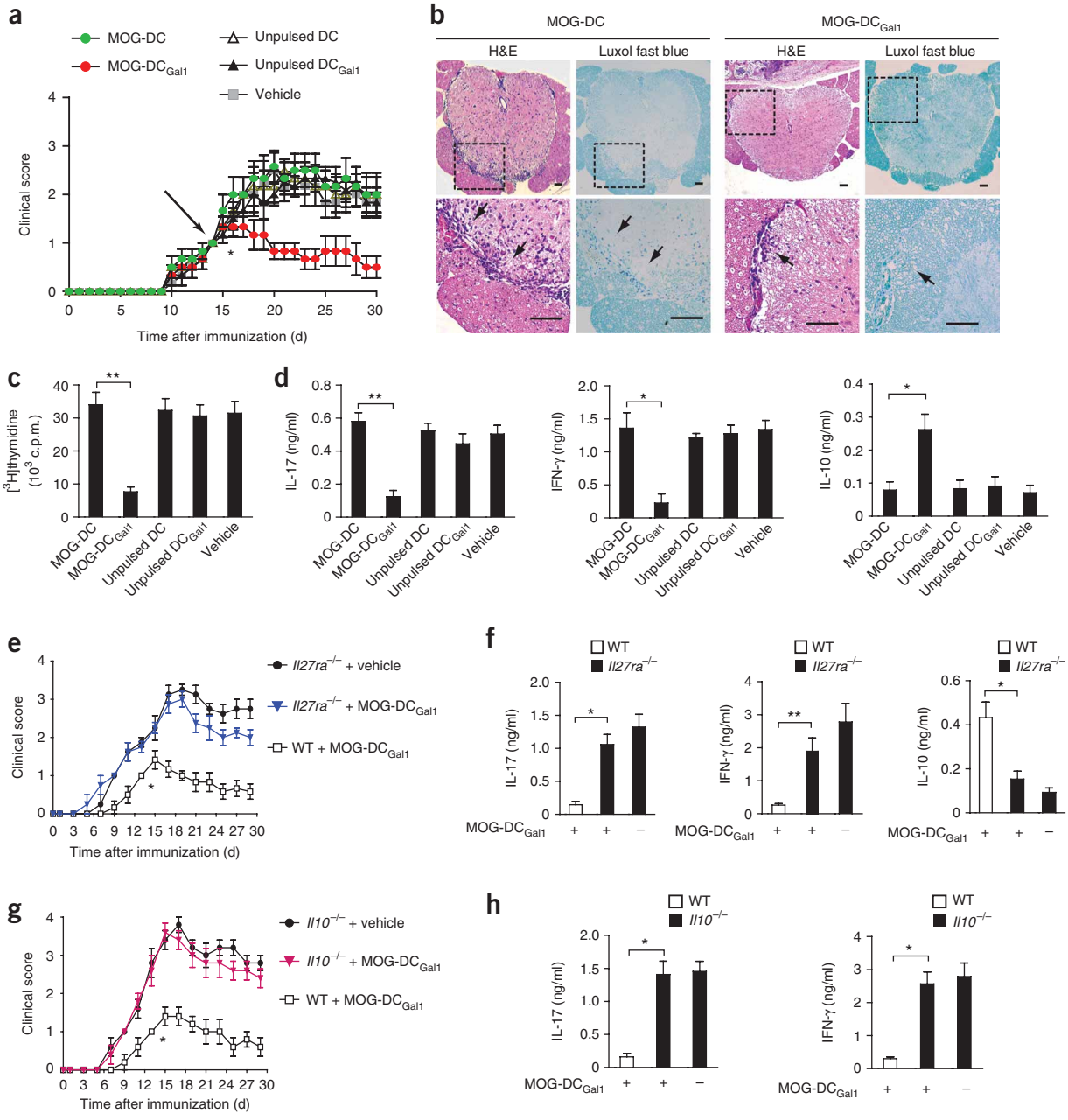

Figure 5 Galectin-1-differentiated DCs suppress T_H-17- and T_H1-mediated neuroinflammation via IL-27 and IL-10. (**a**) Clinical scores of C57BL/6 mice immunized with MOG(35–55) and treated with MOG(35–55)-pulsed BM-DC (MOG-DC) or MOG(35–55)-pulsed BM-DC_{Gal1} (MOG-DC_{Gal1}) or their unpulsed counterparts. Arrow indicates time of DC injection (clinical score, 1). $^*P < 0.01$, versus MOG-DC (Mann-Whitney U-test). Data are from three experiments with five to six mice per group (mean ± s.e.m.). (**b**) Spinal cord sections from the mice described in **a**, stained with hematoxylin and eosin (H&E) or Luxol fast blue. Arrows indicate inflammatory infiltrates and demyelinated areas. Boxes in top row outline areas shown in higher magnification below. Scale bars, 25 μm. Data are representative of three experiments. (**c**,**d**) [^{3}H]thymidine incorporation (**c**) and ELISA of cytokine production (**d**) by lymph node cells obtained 30 d after immunization of the mice described in **a**, then restimulated for 72 h *ex vivo* with MOG(35–55). $^*P < 0.05$ and $^{**}P < 0.01$ (Student's *t*-test). (**e**) Clinical scores of *Il27ra*$^{-/-}$ and wild-type C57BL/6 mice immunized as described in **a**. $^*P < 0.01$, *Il27ra*$^{-/-}$ + MOG-DC_{Gal1} versus WT + MOG-DC_{Gal1} (Mann-Whitney U-test). (**f**) ELISA of cytokine production by lymph node cells collected at day 30 from the mice described in **e** and then restimulated *ex vivo* with MOG(35–55). $^*P < 0.05$ and $^{**}P < 0.01$ (Student's *t*-test). (**g**) Clinical scores of *Il10*$^{-/-}$ and wild-type C57BL/6 mice immunized as described in **a**. $^*P < 0.01$, *Il10*$^{-/-}$ + MOG-DC_{Gal1} versus WT + MOG-DC_{Gal1} (Mann-Whitney U-test). (**h**) ELISA of cytokine production by lymph node cells collected at day 30 from the mice described in **g** and then restimulated *ex vivo* with MOG(35–55). $^*P < 0.01$ (Student's *t*-test). Data are from three (**c**,**d**) or two (**e**–**h**) experiments with five to six mice per group (mean + s.e.m. (**c**,**d**,**f**,**h**) or mean ± s.e.m. (**e**,**g**)).

into *Il10*$^{-/-}$ mice (**Fig. 5g,h** and **Supplementary Table 1c**). These results indicate an essential function for galectin-1 in driving the differentiation of tolerogenic DCs that blunt T_H1 and T_H-17 responses and halt autoimmune neuroinflammation through mechanisms involving IL-27 and IL-10.

Regulation of galectin-1 expression

The observed tolerogenic activity of DC_{Gal1} prompted us to investigate the relevance of endogenous galectin-1 during the progression and resolution of inflammatory disease. For this, we evaluated galectin-1 expression in lymph nodes (draining sites of immunization) and spleens from mice with EAE at various time points after immunization (**Fig. 6a–d**). We found a progressive increase in galectin-1 transcript and protein expression (**Fig. 6a,b**), as well as in the number of galectin-1$^+$ cells (**Supplementary Fig. 8**), in lymph nodes from mice with EAE; these increases peaked during the third week and persisted into the fourth week (recovery phase), reflecting the pace of the clinical disease. Notably, galectin-1 expression also increased over time in the spleens of immunized mice, although increases first became apparent in the second week, peaked at the third week and decreased during the fourth week analyzed (**Fig. 6c,d** and **Supplementary Fig. 8**). CD11c$^+$ DCs purified from spleens at various times of EAE induction had a similar expression profile (**Fig. 6e**). These expression patterns suggest that endogenous galectin-1 may participate in limiting the inflammatory response, thus contributing to disease recovery.

To determine whether endogenous galectin-1 influences the tolerogenic activity of DCs, we investigated the expression of this protein in immature and mature mouse DCs. Bone marrow–derived immature DCs synthesized and secreted large amounts of galectin-1, which decreased substantially after maturation (**Fig. 6f,g**). Given that different profile, we examined the relevant stimuli and signaling pathways that lead to galectin-1 expression on mouse BM-DC (**Supplementary Fig. 9**). We first differentiated bone marrow cells with GM-CSF in the presence of a panel of tolerogenic stimuli, including VIP,

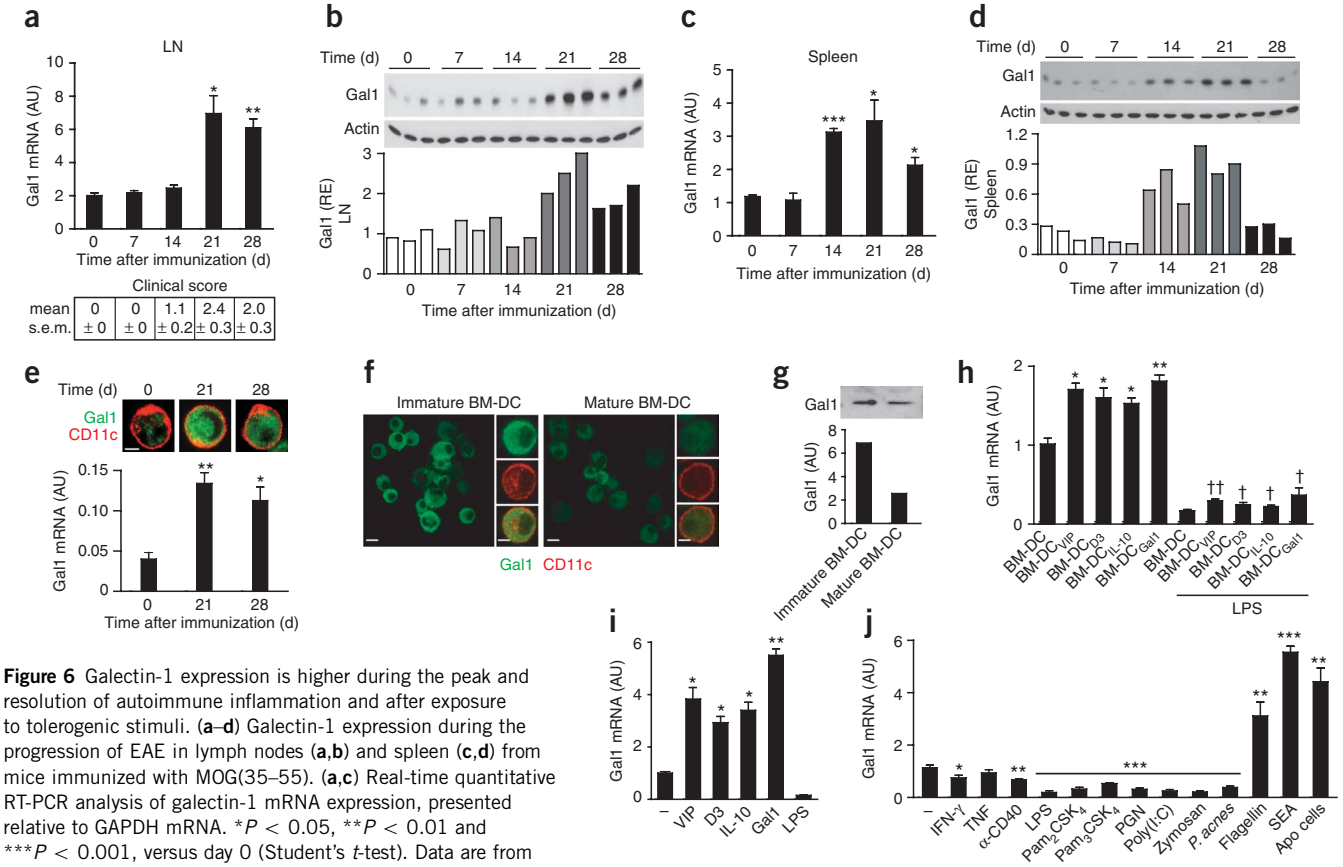

Figure 6 Galectin-1 expression is higher during the peak and resolution of autoimmune inflammation and after exposure to tolerogenic stimuli. (**a–d**) Galectin-1 expression during the progression of EAE in lymph nodes (**a**,**b**) and spleen (**c**,**d**) from mice immunized with MOG(35–55). (**a**,**c**) Real-time quantitative RT-PCR analysis of galectin-1 mRNA expression, presented relative to GAPDH mRNA. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, versus day 0 (Student's *t*-test). Data are from two experiments with five to six mice per group (mean + s.e.m.). (**b**,**d**) Immunoblot analysis of galectin-1 expression (top); below, band intensity relative to that of actin. Each lane represents an individual mouse. Data are representative of two experiments with five to six mice per group. (**e**) Laser confocal microscopy (top) of galectin-1 (green) expression on DCs (CD11c; red) isolated from spleens at various times after EAE induction. Scale bar, 5 μm. Data are representative of two experiments with five to six mice per group. Below, real-time quantitative RT-PCR analysis of galectin-1 mRNA expression in splenic DCs isolated at various times after EAE induction, presented relative to GAPDH mRNA. $*P < 0.05$ and $**P < 0.01$, versus day 0 (Student's *t*-test). Data are from two experiments with five to six mice per group (mean + s.e.m.). (**f**) Laser confocal microscopy of galectin-1 (green) and CD11c (red) in immature and mature mouse BM-DCs. Scale bars, 10 μm (main images) or 5 μm (insets). Data are representative of three experiments. (**g**) Immunoblot analysis of galectin-1 secretion from immature and mature BM-DC. Equal protein loading was checked by Ponceau S staining (data not shown). Data are representative of three experiments. (**h**) Real-time quantitative RT-PCR analysis of galectin-1 mRNA expression in bone marrow cells exposed to GM-CSF and tolerogenic stimuli, including VIP (BM-DC_{VIP}), 1,25-dihydroxyvitamin D_3 (BM-DC_{D3}), IL-10 (BM-DC_{IL-10}) and galectin-1 (BM-DC_{Gal1}), with (right half) or without (left half) further exposure to LPS, presented relative to GAPDH mRNA. $*P < 0.01$ and $**P < 0.001$, versus control DCs; $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$, versus LPS-stimulated DCs (Student's *t*-test). Data are from three experiments (mean + s.e.m.). (**i**) Real-time quantitative RT-PCR analysis of galectin-1 mRNA expression in immature DCs exposed to LPS or tolerogenic stimuli, presented relative to GAPDH mRNA. $*P < 0.01$ and $**P < 0.001$, versus control BM-DC (Student's *t*-test). Data are from three experiments (mean + s.e.m.). (**j**) Real-time quantitative RT-PCR analysis of galectin-1 mRNA expression in immature BM-DC exposed to various stimuli (horizontal axis), presented relative to GAPDH mRNA. Apo cells, apoptotic splenocytes. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, versus control DCs (Student's *t*-test). Data are from three experiments (mean + s.e.m.).

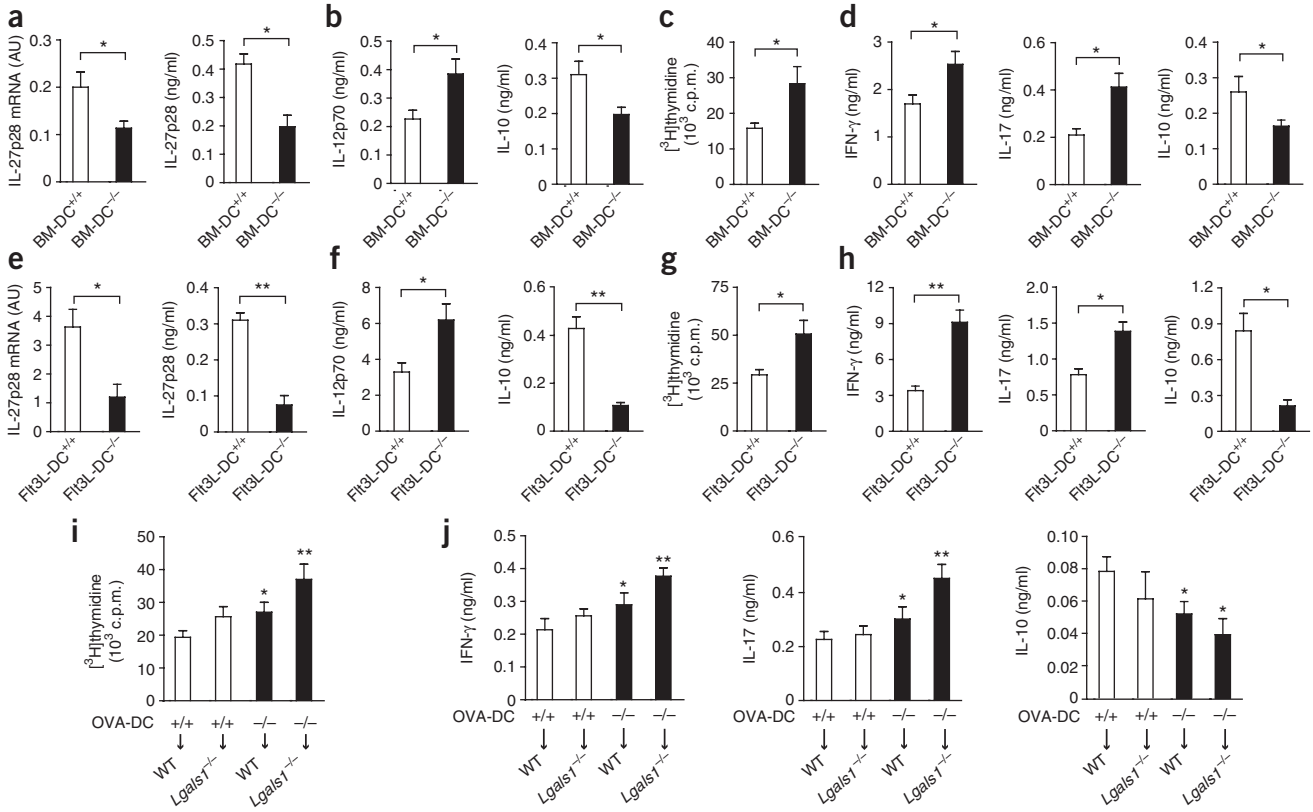

Figure 7 Endogenous galectin-1 'fine tunes' the tolerogenic function of DCs. (**a**) Real-time quantitative RT-PCR analysis of mRNA encoding IL-27p28 and ELISA of IL-27p28 in supernatants of bone marrow–derived *Lgals1*$^{-/-}$ DCs (BM-DC$^{-/-}$) and wild-type DCs (BM-DC$^{+/+}$) differentiated for 8 d with GM-CSF and further matured with LPS; results are presented relative to GAPDH mRNA. *$P < 0.05$ (Student's *t*-test). (**b**) ELISA of cytokines in supernatants of BM-DC$^{-/-}$ or BM-DC$^{+/+}$ differentiated with GM-CSF and further matured with LPS. *$P < 0.05$ (Student's *t*-test). (**c**,**d**) [^{3}H]thymidine incorporation (**c**) and cytokine production (**d**) by allogeneic CD4$^+$ splenocytes (BALB/c) stimulated for 5 d with BM-DC$^{-/-}$ or BM-DC$^{+/+}$ (DC/T cell ratio, 1:10). *$P < 0.05$ (Student's *t*-test). Data in **a**–**d** are from three experiments (mean + s.e.m.). (**e**,**f**) Real-time quantitative RT-PCR analysis (**e**) and ELISA of cytokines (**f**) of splenic DCs obtained from *Lgals1*$^{-/-}$ mice (Flt3L-DC$^{-/-}$) and wild-type mice (Flt3L-DC$^{+/+}$) injected with Flt3L for 9 d and further exposed to LPS, assessed as described in **a**–**d**. (**g**,**h**) [^{3}H]thymidine incorporation (**g**) and cytokine production (**h**) by allogeneic CD4$^+$ splenocytes (BALB/c) stimulated for 5 d with Flt3L-DC$^{-/-}$ or Flt3L-DC$^{+/+}$ (DC/T cell ratio, 1:10). DCs obtained from Flt3L-treated mice in the absence of LPS maturation had similar tendencies (data not shown). *$P < 0.05$ and **$P < 0.01$ (Student's *t*-test; **e**–**h**). Data are from three experiments (mean + s.e.m.; **e**–**h**). (**i**,**j**) Bone marrow–derived *Lgals1*$^{-/-}$ and wild-type DCs differentiated with GM-CSF were pulsed with OVA and transferred into either *Lgals1*$^{-/-}$ or wild-type recipient mice; 7 d after transfer, mice were injected with OVA in CFA, and 1 week later, splenocytes were restimulated *ex vivo* with OVA and analyzed by [^{3}H]thymidine incorporation (**i**) or ELISA of cytokines in supernatants (**j**). There were no detectable changes in splenocytes of wild-type or *Lgals1*$^{-/-}$ mice given unpulsed *Lgals1*$^{-/-}$ or wild-type BM-DC (data no shown). *$P < 0.05$ and **$P < 0.01$, versus wild-type BM-DCs transferred to wild-type mice (Student's *t*-test). Data are from three experiments with four to five mice per group (mean + s.e.m.).

1,25-dihydroxyvitamin D_3, IL-10 and galectin-1 itself. All tolerogenic stimuli induced substantial increases, albeit to varying extents, in galectin-1 transcript and protein expression (**Fig. 6h** and **Supplementary Fig. 10a**). This effect was more pronounced when we first differentiated BM-DC with GM-CSF and subsequently exposed immature BM-DC to tolerogenic stimuli (**Fig. 6i** and **Supplementary Fig. 10b**). Finally, we exposed immature BM-DCs to proinflammatory cytokines, CD40 crosslinking and agonists of Toll-like receptors or C-type lectin receptors. Exposure to most Toll-like receptor agonists (LPS, Pam_2CSK_4, Pam_3CSK_4, peptidoglycan, poly(I:C), zymosan and heat-killed *Propionibacterium acnes*), as well as proinflammatory cytokines (IFN-γ and tumor necrosis factor) and CD40 crosslinking, resulted in less galectin-1 expression (**Fig. 6j**). However, exposure of immature BM-DC to *Schistosoma mansoni* egg antigen (SEA) or to flagellin led to much more galectin-1 expression (**Fig. 6j**). Moreover, culture of immature BM-DC together with apoptotic splenocytes, an interaction that favors the induction of tolerogenic DCs[5], also resulted in the synthesis of large amounts of galectin-1 (**Fig. 6j** and **Supplementary Fig. 10c**). Thus, tolerogenic stimuli can selectively upregulate galectin-1 expression during the differentiation or maturation of mouse BM-DC. To further delineate the signaling pathways that lead to galectin-1 expression, we exposed immature BM-DC to SEA (the most potent stimulus augmenting galectin-1 synthesis) in the presence or absence of specific signaling inhibitors. Inhibition of the mitogen-activated protein kinases Erk1-Erk2 or Jnk abrogated SEA-induced upregulation of galectin-1, but inhibition of the kinases PI(3)K and Akt, the mitogen-activated protein kinase p38, NF-κB or STAT3 did not (**Supplementary Fig. 11**).

Endogenous galectin-1 'fine tunes' the tolerogenic function of DCs

Given the selective upregulation of endogenous galectin-1 by tolerogenic stimuli, we next examined the effects of *Lgals1* deletion on DC function by differentiating bone marrow cells from *Lgals1*$^{-/-}$ and wild-type mice in the presence of GM-CSF. *In vitro*–differentiated *Lgals1*$^{-/-}$ and wild-type BM-DC had a similar cell surface phenotype (data not shown). However, after maturation, BM-DC devoid of

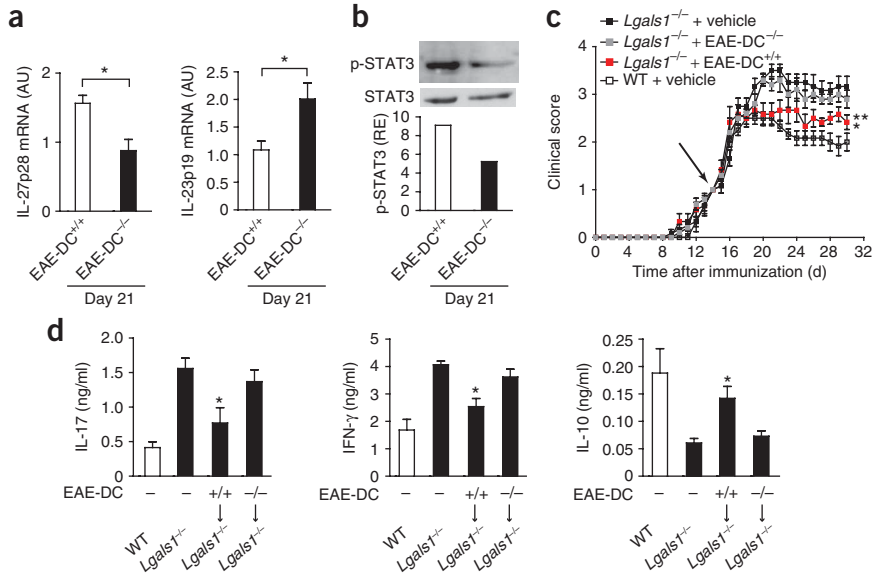

Figure 8 Galectin-1-sufficient tolerogenic DCs contribute to the resolution of EAE. (**a**) Real-time quantitative RT-PCR analysis of mRNA encoding IL-27p28 and IL-23p19 in CD11c$^+$ DCs purified from the spleens of *Lgals1*$^{-/-}$ mice (EAE-DC$^{-/-}$) or wild-type mice (EAE-DC$^{+/+}$) at day 21 after immunization with MOG(35–55). Results are presented relative to GAPDH mRNA. *$P < 0.05$ (Student's *t*-test). Data are from three experiments (mean + s.e.m.). (**b**) Immunoblot analysis of phosphorylated STAT3 in the cells analyzed in **a**; below, band intensity relative to that of STAT3. Data are representative of three experiments. (**c**) Disease progression of *Lgals1*$^{-/-}$ mice given CD11c$^+$ DCs purified from spleens of *Lgals1*$^{-/-}$ or wild-type mice 21 d after EAE induction; cells were adoptively transferred at the day of disease onset of recipient mice (arrow). *$P < 0.05$, versus *Lgals1*$^{-/-}$ + EAE-DC$^{-/-}$; **$P < 0.01$, versus *Lgals1*$^{-/-}$ + vehicle (Mann-Whitney U-test). Data are from two experiments with five to six mice per group (mean ± s.e.m.). (**d**) ELISA of cytokine production by lymph node cells obtained 30 d after immunization of the mice in **c**, then restimulated for 72 h *ex vivo* with MOG(35–55). *$P < 0.05$, versus *Lgals1*$^{-/-}$ + EAE-DC$^{-/-}$ and *Lgals1*$^{-/-}$ + vehicle (Student's *t*-test). Data are from two experiments with five to six mice per group (mean + s.e.m.).

galectin-1 expressed less IL-27p28 transcript and protein (**Fig. 7a**) and synthesized more IL-12p70 and less IL-10 (**Fig. 7b**) than did its wild-type counterparts. Consistent with those findings, allogeneic CD4$^+$ T cells stimulated with galectin-1-deficient BM-DC had more robust proliferation, secreted more IFN-γ and IL-17 and synthesized less IL-10 than did those primed with wild-type BM-DC (**Fig. 7c,d**).

Given the intrinsic heterogeneity of DCs[26], we then investigated whether the regulatory effect of endogenous galectin-1 could be generalized to *in vivo*–differentiated DCs. We purified CD11c$^+$ DCs from spleens of *Lgals1*$^{-/-}$ and wild-type mice after administration of the ligand for the receptor tyrosine kinase Flt3 (Flt3L)[27]. Although we found no substantial difference in cell surface phenotypic markers (data not shown), we detected much less IL-27p28 transcript and protein in Flt3L-induced *Lgals1*$^{-/-}$ DCs than in their wild-type counterparts (**Fig. 7e**). This effect was accompanied by more secretion of IL-12p70 and less secretion of IL-10 from Flt3L-induced *Lgals1*$^{-/-}$ DCs than from wild-type DCs after maturation (**Fig. 7f**). Moreover, *Lgals1*$^{-/-}$ DCs had greater allostimulatory capacity than wild-type DCs when isolated from Flt3L-treated mice (**Fig. 7g,h**).

To analyze the immunogenicity of *Lgals1*$^{-/-}$ DCs *in vivo*, we pulsed GM-CSF-differentiated *Lgals1*$^{-/-}$ or wild-type BM-DC with OVA and adoptively transferred these cells into *Lgals1*$^{-/-}$ or wild-type recipient mice. At 7 d after immunization, we injected mice with OVA in CFA. Splenocytes from wild-type or *Lgals1*$^{-/-}$ mice given antigen-pulsed *Lgals1*$^{-/-}$ BM-DC showed enhanced T cell proliferation, more production of IFN-γ and IL-17 and less synthesis of IL-10 than did cells from mice that received OVA-pulsed wild-type BM-DC (**Fig. 7i,j**). Collectively these data suggest a regulatory function for endogenous galectin-1 in 'fine tuning' the immunogenic capacity of DCs.

Galectin-1-expressing DCs contribute to EAE resolution

To study whether endogenously regulated galectin-1, specifically on DCs, contributes to the resolution of autoimmune inflammation, we immunized *Lgals1*$^{-/-}$ and wild-type mice with MOG(35–55) and purified CD11c$^+$ DCs from spleens at the peak of the disease (day 21). We analyzed the expression of IL-27 and IL-23 on these cells, which have opposing functions on T_H-17 differentiation and EAE progression[12,24]. Consistent with a greater frequency of tolerogenic DCs necessary for limiting clinical severity and initiating disease recovery, DCs isolated from wild-type mice at the peak of EAE expressed substantial amounts of IL-27p28 but limited amounts of IL-23p19 mRNA (**Fig. 8a**), which accompanied high expression of galectin-1 on these cells (**Fig. 6e**). Galectin-1 deficiency altered this regulatory profile, as *Lgals1*$^{-/-}$ DCs had lower expression of IL-27p28, higher expression of IL-23p19 and less STAT3 phosphorylation than did wild-type DCs (**Fig. 8a,b**).

To further delineate the tolerogenic function of galectin-1-expressing DCs during the resolution of inflammation, we isolated DCs from spleens of wild-type or *Lgals1*$^{-/-}$ mice at day 21 of EAE and adoptively transferred these cells into *Lgals1*$^{-/-}$ mice at the day of disease onset (**Supplementary Fig. 12**). In keeping with a regulatory function for endogenous galectin-1, disease was more severe in *Lgals1*$^{-/-}$ mice than in wild-type mice[19]. Galectin-1-sufficient tolerogenic DCs (isolated at day 21) successfully restored T cell tolerance and contributed to the resolution of autoimmune inflammation when adoptively transferred into *Lgals1*$^{-/-}$ mice (**Fig. 8c,d** and **Supplementary Table 2**). This effect was associated with less secretion of IL-17 and IFN-γ and more secretion of IL-10 by lymph node cells from *Lgals1*$^{-/-}$ mice that received galectin-1-sufficient DCs relative to that of *Lgals1*$^{-/-}$ mice that received galectin-1-deficient DCs (**Fig. 8d**). Thus, endogenously regulated galectin-1, particularly on DCs, contributes to the resolution of autoimmune neuroinflammation by driving the generation of IL-27-producing tolerogenic DCs at late stages of the ongoing inflammatory response.

DISCUSSION

Efforts to 'decode' the glycosylation 'signature' of processes in cells of the immune system have demonstrated substantial changes in *N*-glycan and *O*-glycan structures during T cell activation and differentiation[19,28,29]. These alterations have also been detected during the course of DC differentiation and maturation[22], which suggests that protein-glycan interactions may serve a decisive function in the control of responsiveness and tolerance of cells of the immune system[15,16]. Here we have identified an essential function for galectin-1

in the generation of human and mouse tolerogenic DCs. Galectin-1-differentiated DCs acquired a distinctive regulatory profile, promoted T cell tolerance and terminated autoimmune neuroinflammation through mechanisms involving IL-27 and IL-10. In addition, we have demonstrated a role for endogenous galectin-1 in 'fine tuning' the tolerogenic function of DCs during the resolution of autoimmune inflammation.

Emerging evidence indicates that endogenous glycan-binding proteins, particularly C-type lectin receptors, may serve as signaling molecules that relay pathogen or tumor information into distinct DC-differentiation programs[30–33]. Dectin-1, a C-type lectin receptor that recognizes β-glucan structures on yeasts, can signal DCs either to drive the differentiation of T_H-17 cells[30] or to promote the population expansion of tolerogenic DCs[31]. Similarly, engagement of P-selectin or expression of the C-type lectin receptor Dcir triggers an inhibitory signal that limits DC functionality[32,33]. Moreover, tolerogenic DCs can suppress T cell activation through glycosylation-dependent interactions mediated by CD45 and macrophage galactose-type lectin[29]. Thus, distinct protein-glycan systems may have evolved as 'on-off' switches that control the induction of tolerogenic versus inflammatory DCs.

The mechanisms underlying the anti-inflammatory activity of galectin-1 remain poorly understood. Although this protein modulates the survival of effector T cells[19,34,35], skews the balance of T helper cytokines[17–20,36,37] and controls transendothelial T cell migration[38], these mechanisms do not broadly support the immunosuppressive effects observed at early and late phases of the inflammatory response[16]. In a search for alternative mechanisms, we have identified an immunoregulatory circuit initiated by galectin-1 on DCs that drives IL-27-dependent, IL-10-mediated T cell tolerance. Although studies have indicated that DCs engineered to overexpress galectin-1 or exposed to high concentrations of this protein (20 μM) acquire a mature cell surface phenotype[39,40], we have demonstrated here that galectin-1 at lower concentrations can induce tolerogenic activity in human and mouse DCs when added during the differentiation or maturation process. One explanation for these apparent discrepancies could be a bifunctional role for galectin-1, acting either as a tolerogenic or immunogenic signal depending on its relative concentration or the prevalence of immunosuppressive versus inflammatory microenvironments. Nevertheless, the bimodal paradigm of fully mature DCs eliciting adaptive immunity or immature DCs acting as promoters of T cell tolerance has been challenged, which indicates that phenotypic maturation itself is not a hallmark of immunogenic rather than tolerogenic DCs[2]. In this context, we found that exposure to galectin-1 during the maturation process induced the generation of DCs with a typically mature cell surface phenotype but dominant regulatory potential. In agreement with that, progesterone-regulated galectin-1 restores tolerance in failing pregnancies, and this effect correlates with the population expansion of T_{reg} cells and the appearance of uterine cells with a regulatory DC phenotype[20].

Several mechanisms have been proposed to explain how DCs drive T helper–differentiation programs[2]. However, the nature of specialized DCs that selectively dampen T_H1-, T_H2- or T_H-17-driven effector immunity is uncertain. In this context, studies have emphasized a dominant function for IL-27-producing DCs in the generation of IL-10$^+$Foxp3$^-$ type 1 T_{reg} cells[11]. Given that IL-27-producing DCs may be generated by close contact with inducible CD4$^+$CD25$^+$Foxp3$^+$ T_{reg} cells[11], which are a chief source of galectin-1 (ref. 41), we postulate that this endogenous lectin might represent an elusive immunosuppressive signal that links T_{reg} cell–induced immunosuppression, IL-27-producing tolerogenic DCs and IL-10-producing type 1 T_{reg} cells. Notably, mice that lack IL-27Rα or IL-10 develop exacerbated EAE owing to hyper-T_H-17 or hyper-T_H1 responses[42,43], and *Lgals1*$^{-/-}$ mice completely recapitulate those phenotypes[19]. In agreement with that, the tolerogenic effect of DC_{Gal1} was almost completely eliminated in *Il27ra*$^{-/-}$ and *Il10*$^{-/-}$ mice, thus substantiating the function of IL-27 and IL-10 in promoting galectin-1-mediated activities. Notably, galectin-1 expression increased during the peak and recovery phases of EAE, like the expression of other regulatory signal molecules such as programmed death 1 and its ligand, PD-L1 (ref. 44), which suggests that this glycan-binding protein may contribute to tempering inflammatory responses and driving the resolution of autoimmune pathology. In support of that idea, galectin-1 expression increased after exposure to tolerogenic stimuli but was rapidly downregulated in response to DC maturation signals, similar to the expression of CCR9, a chemokine receptor that defines tolerogenic plasmacytoid DCs[45]. These contrasting effects of tolerogenic versus inflammatory stimuli on galectin-1 expression might offer an alternative explanation for the plasticity of DCs in their response to Toll-like receptor or C-type lectin receptor signals and in driving divergent T helper responses[46,47]. However, given the intrinsic differences of DC subtypes[26], caution should be taken before any attempt to generalize the tolerogenic effect of galectin-1. In this context, our study focused on GM-CSF-differentiated human and mouse DCs, DCs mobilized *in vivo* after administration of Flt3L and galectin-1-sufficient DCs whose populations were expanded during the peak and resolution of the inflammatory disease. How endogenous galectin-1 promotes IL-27^{hi} regulatory DCs still remains to be elucidated, although in keeping with its biochemical features[16], this lectin might function intracellularly or might be secreted from DCs and act in an autocrine or paracrine way to favor a tolerogenic profile.

Galectin-1 recognizes multiple galactose-β1-4-*N*-acetylglucosamine (LacNAc) units present on the branches of *N*-linked or *O*-linked glycans[16]. Therefore, the regulated expression of glycosyltransferases[22], which creates poly-LacNAc ligands, may determine susceptibility to galectin-1. Consistent with a regulatory function for galectin-glycan lattices, interruption of β1,6-branching on *N*-glycans or blockade of poly-LacNAc synthesis results in an altered sensitivity to cytokine signaling and a lower threshold for the activation of antigen-presenting cells[48,49]. Moreover, ligation of Tim-3, a receptor specific for galectin-9, induces divergent functions in antigen-presenting cells and T cells that lead to initiation or termination of T_H1 immunity[50], whereas galectin-1 and galectin-3 trigger distinct signaling events that modulate the function of peritoneal macrophages[51,52]. Thus, galectin-glycan interactions may have evolved to regulate antigen-presenting cell homeostasis and to control their activation and signaling.

Our findings have demonstrated an essential role for endogenous galectin-1 in 'fine tuning' the tolerogenic function of DCs, similar to that of other regulatory signals such as STAT3, SOCS1 and HDAC11 (refs. 21,53). Our results have also identified an immunoregulatory circuit linking galectin-1 signaling, IL-27-producing tolerogenic DCs and IL-10-secreting T_{reg} cells. Strategies that manipulate this circuit in either direction (stimulation or blockade) may be able to influence immune tolerance versus activation, a crucial 'decision' with broad implications in autoimmunity, infection, transplantation and cancer.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Accession codes. UCSD-Nature Signaling Gateway (http://www.signaling-gateway.org): A001243 and A002911.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

J.M.I. designed and did all the experiments and contributed to manuscript preparation; D.O.C. contributed to immunohistochemistry, immunoblot analysis and confocal microscopy; G.A.B. contributed to EAE experiments; M.A.T. contributed to immunoblot and *in vivo* assays; M.S. contributed to real-time RT-PCR; M.E.V. contributed to *in vivo* assays; J.R.G. provided essential reagents and intellectual support; and G.A.R. supervised the work, designed the experiments and wrote the manuscript.

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1. Guermonprez, P., Valladeau, J., Zitvogel, L., Thery, C. & Amigorena, S. Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol.* **20**, 621–667 (2002).
2. Reis e Sousa, C. Dendritic cells in a mature age. *Nat. Rev. Immunol.* **6**, 476–483 (2006).
3. Steinman, R.M., Hawiger, D. & Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **21**, 685–711 (2003).
4. Morelli, A.E. & Thomson, A.W. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat. Rev. Immunol.* **7**, 610–621 (2007).
5. Skoberne, M. *et al.* The apoptotic-cell receptor CR3, but not αvβ5, is a regulator of human dendritic-cell immunostimulatory function. *Blood* **108**, 947–955 (2006).
6. Zhang, M. *et al.* Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. *Nat. Immunol.* **5**, 1124–1133 (2004).
7. Svensson, M., Maroof, A., Ato, M. & Kaye, P.M. Stromal cells direct local differentiation of regulatory dendritic cells. *Immunity* **21**, 805–816 (2004).
8. Wakkach, A. *et al.* Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation *in vivo*. *Immunity* **18**, 605–617 (2003).
9. Chorny, A. *et al.* Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. *Proc. Natl. Acad. Sci. USA* **102**, 13562–13567 (2005).
10. Penna, G. *et al.* 1,25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. *J. Immunol.* **178**, 145–153 (2007).
11. Awasthi, A. *et al.* A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat. Immunol.* **8**, 1380–1389 (2007).
12. Kastelein, R.A., Hunter, C.A. & Cua, D.J. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu. Rev. Immunol.* **25**, 221–242 (2007).
13. Stumhofer, J.S. *et al.* Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol.* **8**, 1363–1371 (2007).
14. Fitzgerald, D.C. *et al.* Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27–stimulated T cells. *Nat. Immunol.* **8**, 1372–1379 (2007).
15. van Kooyk, Y. & Rabinovich, G.A. Protein-glycan interactions in the control of innate and adaptive immune responses. *Nat. Immunol.* **9**, 593–601 (2008).
16. Rabinovich, G.A. & Toscano, M.A. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat. Rev. Immunol.* **9**, 338–352 (2009).
17. Rubinstein, N. *et al.* Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. *Cancer Cell* **5**, 241–251 (2004).
18. Juszczynski, P. *et al.* The AP1-dependent secretion of galectin-1 by Reed Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. *Proc. Natl. Acad. Sci. USA* **104**, 13134–13139 (2007).
19. Toscano, M.A. *et al.* Differential glycosylation of T_H1, T_H2 and T_H-17 effector cells selectively regulates susceptibility to cell death. *Nat. Immunol.* **8**, 825–834 (2007).
20. Blois, S.M. *et al.* A pivotal role for galectin-1 in fetomaternal tolerance. *Nat. Med.* **13**, 1450–1457 (2007).
21. Rabinovich, G.A., Gabrilovich, D. & Sotomayor, E.M. Immunosuppressive strategies that are mediated by tumor cells. *Annu. Rev. Immunol.* **25**, 267–296 (2007).
22. Bax, M. *et al.* Dendritic cell maturation results in pronounced changes in glycan expression affecting recognition by siglecs and galectins. *J. Immunol.* **179**, 8216–8224 (2007).
23. Kotera, Y., Shimizu, K. & Mule, J.J. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization. *Cancer Res.* **61**, 8105–8109 (2001).
24. Steinman, L. A brief history of T_H-17, the first major revision in the T_H1/T_H2 hypothesis of T cell-mediated tissue damage. *Nat. Med.* **13**, 139–145 (2007).
25. Stromnes, I.M., Cerretti, L.M., Liggitt, D., Harris, R.A. & Goverman, J.M. Differential regulation of central nervous system autoimmunity by T_H1 and T_H-17 cells. *Nat. Med.* **14**, 337–342 (2008).
26. Villadangos, J.A. & Schnorrer, P. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets *in vivo*. *Nat. Rev. Immunol.* **7**, 543–555 (2007).
27. Waskow, C. *et al.* The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat. Immunol.* **9**, 676–683 (2008).
28. Comelli, E.M. *et al.* Activation of murine $CD4^+$ and $CD8^+$ T lymphocytes leads to dramatic remodeling of N-linked glycans. *J. Immunol.* **177**, 2431–2440 (2006).
29. van Vliet, S.J., Gringhuis, S.I., Geijtenbeek, T.B. & van Kooyk, Y. Regulation of effector T cells by antigen-presenting cells via interaction of the C-type lectin MGL with CD45. *Nat. Immunol.* **7**, 1200–1208 (2006).
30. LeibundGut-Landmann, S. *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat. Immunol.* **8**, 630–638 (2007).
31. Dillon, S. *et al.* Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* **116**, 916–928 (2006).
32. Urzainqui, A. *et al.* Functional role of P-selectin glycoprotein ligand 1/P-selectin interaction in the generation of tolerogenic dendritic cells. *J. Immunol.* **179**, 7457–7465 (2007).
33. Fujikado, N. *et al.* Dcir deficiency causes development of autoimmune diseases in mice due to excess expansion of dendritic cells. *Nat. Med.* **14**, 176–180 (2008).
34. Stillman, B.N. *et al.* Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J. Immunol.* **176**, 778–789 (2006).
35. Motran, C.C. *et al.* Galectin-1 functions as a Th2 cytokine that selectively induces Th1 apoptosis and promotes Th2 function. *Eur. J. Immunol.* **38**, 3015–3027 (2008).
36. Stowell, S.R. *et al.* Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. *J. Immunol.* **180**, 3091–3102 (2008).
37. van der Leij, J. *et al.* Strongly enhanced IL-10 production using stable galectin-1 homodimers. *Mol. Immunol.* **44**, 506–513 (2007).
38. Norling, L.V., Sampaio, A.L., Cooper, D. & Perretti, M. Inhibitory control of endothelial galectin-1 on in vitro and in vivo lymphocyte trafficking. *FASEB J.* **22**, 682–690 (2008).
39. Perone, M.J. *et al.* Transgenic galectin-1 induces maturation of dendritic cells that elicit contrasting responses in naive and activated T cells. *J. Immunol.* **176**, 7207–7220 (2006).
40. Fulcher, J.A. *et al.* Galectin-1-matured human monocyte-derived dendritic cells have enhanced migration through extracellular matrix. *J. Immunol.* **177**, 216–226 (2006).
41. Garin, M.I. *et al.* Galectin-1: a key effector of regulation mediated by $CD4^+CD25^+$ T cells. *Blood* **109**, 2058–2065 (2007).
42. Batten, M. *et al.* Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17–producing T cells. *Nat. Immunol.* **7**, 929–936 (2006).
43. Bettelli, E. *et al.* IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J. Immunol.* **161**, 3299–3306 (1998).
44. Salama, A.D. *et al.* Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. *J. Exp. Med.* **198**, 71–78 (2003).
45. Hadeiba, H. *et al.* CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. *Nat. Immunol.* **9**, 1253–1260 (2008).
46. Kane, C.M. *et al.* Helminth antigens modulate TLR-initiated dendritic cell activation. *J. Immunol.* **173**, 7454–7461 (2004).
47. Boonstra, A. *et al.* Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential Toll-like receptor ligation. *J. Exp. Med.* **6**, 101–109 (2003).
48. Partridge, E.A. *et al.* Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. *Science* **306**, 120–124 (2004).
49. Togayachi, A. *et al.* Polylactosamine on glycoproteins influences basal levels of lymphocyte and macrophage activation. *Proc. Natl. Acad. Sci. USA* **104**, 15829–15834 (2007).
50. Anderson, A.C. *et al.* Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. *Science* **318**, 1141–1143 (2007).
51. Barrionuevo, P. *et al.* A novel function of galectin-1 at the cross-road of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. *J. Immunol.* **178**, 436–445 (2007).
52. MacKinnon, A.C. *et al.* Regulation of alternative macrophage activation by galectin-3. *J. Immunol.* **180**, 2650–2658 (2008).
53. Villagra, A. *et al.* The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. *Nat. Immunol.* **10**, 92–100 (2009).

ONLINE METHODS

Mice. *Lgals1*$^{-/-}$ mice (C57BL/6) were provided by F. Poirier; *Il27ra*$^{-/-}$ mice (C57BL/6) were provided by C.J. Saris; and *Il10*$^{-/-}$ mice (C57BL/6) were from the Jackson Laboratory. Mice were bred in the animal facilities of the Institute of Biology and Experimental Medicine and the Faculty of Exact and Natural Sciences (University of Buenos Aires). Protocols were approved by the respective Institutional Review Boards.

Preparation of recombinant galectin-1. Recombinant galectin-1 was obtained as described[19,51]. As cellular responses might be confounded by LPS contamination[54], LPS was carefully removed by Detoxi-Gel endotoxin-removing gel (Pierce), followed by analysis with a Gel Clot Limulus test (<0.5 IU/mg; Cape Code).

Generation of human and mouse DCs. To obtain human DCs, peripheral blood mononuclear cells were isolated from buffy coats of healthy donors by Ficoll-Hypaque Plus (GE Healthcare). These studies were approved by the Institutional Review Board of Hospital de Clínicas 'José de San Martín'. Monocytes were purified by a discontinuous Percoll gradient (GE Healthcare) and positive selection with the Monocyte Isolation kit (purity, >90%; Miltenyi Biotec). Monocytes were cultured in RPMI-1640 complete medium supplemented with 10% (vol/vol) heat-inactivated FCS, gentamicin (40 μg/ml), 2-mercaptoethanol (50 μM) and L-glutamine (2 mM; all from Gibco) containing IL-4 (5 ng/ml; Sigma) and recombinant human GM-CSF (35 ng/ml; Sigma) with or without galectin-1 (0.3-3 μM). In some experiments, iDCs were exposed for 24 h to LPS (1 μg/ml; from the *Escherichia coli* 0111:B4 strain; Sigma) with or without galectin-1.

For the generation of mouse DCs, bone marrow cells were incubated for 8 d in complete DMEM with recombinant mouse GM-CSF (20 ng/ml; BD Biosciences) or 10% conditioned medium from GM-CSF-producing J588L cells with or without galectin-1 (0.3–3 μM). Control iDC or iDC_{Gal1} were stimulated for 48 h with LPS. In some experiments, bone marrow cells or iDCs were exposed to VIP (10 nM; Calbiochem), 1,25-dihydroxyvitamin D_3 (10 nM; Sigma), IL-10 (50 ng/ml; R&D Systems) or recombinant galectin-1 (3 μM) with or without LPS stimulation. The iDCs were also exposed to IFN-γ (50 ng/ml; R&D), tumor necrosis factor (20 ng/ml; Sigma), agonistic antibody to CD40 (10 μg/ml; HM40-3; BD Biosciences), Pam_2CSK_4 (100 ng/ml; Invivogen), Pam_3CSK_4 (1 μg/ml; Invivogen), *Bacillus subtilis* peptidoglycan (10 μg/ml; Invivogen), poly(I:C) (10 μg/ml; Invivogen), zymosan (10 μg/ml; Invivogen), heat-killed *P. acnes* (20 μg/ml; van Kampen Group), *B. subtilis* flagellin (200 ng/ml; Invivogen), endotoxin-free SEA (50 μg/ml) or *Lgals1*$^{-/-}$ apoptotic splenocytes (irradiated with 12,000 rads), which were separated by sorting in a FACSAria (BD Biosciences) before DC analysis. For analysis of signaling pathways, iDC were incubated with SEA with or without inhibitors of Jak2-STAT3 (2.5 μM AG490; Calbiochem), Jnk-SAP (20 μM SP600125; Calbiochem), Erk1-Erk2 (5 μM U0126; Sigma), PI(3)K-Akt (2 μM Ly294002; Sigma), p38 (10 μM SB202190; Calbiochem) or NF-κB (1 μM BAY11-7082; Sigma). The NF-κB p50-p65 Transcription Factor assay (Millipore) was used for analysis of NF-κB DNA-binding activity. *In vivo*–differentiated CD11c$^+$ DCs were obtained from spleens (purity, >96%) with MicroBeads (MACS; Miltenyi Biotec) after 9 d of administration of 10 μg human recombinant Flt3L (PeproTech)[27].

Galectin-1 binding and segregation assays. Cells were incubated for 1 h at 4 °C with biotinylated galectin-1 with or without lactose or sucrose as described[19]. Cells were then incubated for 45 min at 4 °C with FITC-streptavidin (Pierce) and were analyzed in a FACSAria (BD Biosciences). For segregation assays, iDCs were treated with galectin-1 for 1 h, then were fixed and were incubated for 1 h with monoclonal anti–human CD43 (8.4 μg/ml; DF-T1; Dako) or monoclonal anti–human CD45 (14.5 μg/ml; 2B11; Dako) as described[19]. Cells were analyzed on a Nikon laser confocal microscope (Eclipse E800).

Flow cytometry. Cells were incubated for 30 min at 4 °C with FITC- and phycoerythrin (PE)-labeled monoclonal antibodies (all from BD Biosciences). Human cells were stained with the following monoclonal antibodies: FITC–anti-CD1a (HI149), PE–anti-CD14 (M5E2), FITC–anti-CD86 (2331-FUN-1), FITC–anti-HLA-DR (G46-6) and PE–anti-CD83 (HB15e). Mouse cells were stained with the following monoclonal antibodies: PE–anti-CD11c (HL3), FITC–anti-CD40 (HM40-3), PE–anti-I-A^b (AF6-120.1), PE–anti-H-$2K^b$ (AF6-88.5), FITC–anti-CD80 (16-10A1), FITC–anti-CD86 (GL1), FITC–anti-Gr1 (RB6-8C5) and FITC–anti-CD45RB (16A). For intracellular staining, cells were made permeable with Perm2 solution (BD Biosciences) and were stained with a Foxp3-specific human antibody (PCH101; eBioscience) or mouse antibody (FJK-16s; eBioscience). For endocytosis assays, cells were incubated at 37 °C or 4 °C with FITC-OVA (300 μg/ml; Sigma) and were analyzed on a FACSAria (BD Biosciences).

Cytokine assays. Human and mouse IL-12p70, IL-10, IFN-γ, IL-4, IL-6 and TGF-β_1 ELISA sets were from BD Biosciences. Mouse IL-17 and IL-27p28 ELISA kits were from R&D Systems.

Real-time quantitative RT-PCR. SYBR Green PCR Master Mix (Applied Biosystem) was used with an ABI PRISM 7500 Sequence Detection Software (Applied Biosystem). Primer sequences are in **Supplementary Table 3**.

Immunoblot, immunohistochemistry and confocal microscopy. Immunoblot analysis was done as described[17]. Equal amounts of protein were resolved by SDS-PAGE, blotted onto nitrocellulose membranes (GE Healthcare) and probed with anti-STAT3 (C-20), antibody to phosphorylated STAT3 (B-7), anti-IκBα (C-21), anti-Erk (C-14), antibody to phosphorylated Erk (E-4), anti-p38 (A-12), antibody to phosphorylated p38 (D-8), anti-c-Jun (H-79), antibody to phosphorylated c-Jun (ser63/73-R) or anti-actin (I-19; all from Santa Cruz), anti-Akt (9272; Cell Signaling), antibody to phosphorylated Akt (9271S; Cell Signaling), anti-IL-27-EBI3 (355022; R&D) or immunoglobulin G rabbit anti-galectin-1 (1.5 μg/ml) obtained as described[17,18,20]. Immunohistochemistry was done as described[18] with the Vectastain Elite ABC kit (Vector). For immunofluorescence, cells were fixed, were made permeable with Perm-2 solution (BD Biosciences) and were stained with immunoglobulin G anti-galectin-1 (32 μg/ml) followed by FITC-labeled anti–rabbit immunoglobulin G (Santa Cruz) and PE-anti-CD11c (20 μg/ml; HL-3; BD Biosciences). Cells were analyzed on a Nikon laser confocal microscope (Eclipse E800).

Allogeneic MLRs. Human CD4$^+$ T cells were purified by negative selection with CD4$^+$ RosetteSep reagent (StemCell Technol) and 1×10^5 cells were cultured for 5 d with allogeneic DCs or DC_{Gal1} (irradiated with 3,000 rads) at various DC/T cell ratios. In some experiments, allogeneic CD4$^+$ cells were purified by cell sorting (FACSAria; BD Biosciences) and their regulatory capacity was studied on subsequent MLR cultures in the presence or absence of IL-10-neutralizing antibody (JES3-9D7; BD Biosciences). For examination of the regulatory function of LPS-DC_{Gal1}, allogeneic CD4$^+$ cells (2×10^5) were cultured for 5 d with LPS-matured, fully competent DCs (1×10^4) in the presence or absence of LPS-DC_{Gal1}.

Mouse naive CD4$^+$ cells were isolated from spleens of wild-type (BALB/c) mice with the MagCellect Isolation kit (R&D Systems) and 2×10^5 cells were cultured for 5–6 d together with BM-DC, BM-DC_{Gal1} or *Lgals1*$^{-/-}$ BM-DC (C57BL/6; irradiated with 3,000 rads) at various DC/T cell ratios. Neutralizing antibodies to IL-27p28 (AF1834; R&D), TGF-β (1D11; R&D) or IL-10 receptor (1B1.3a; BD Biosciences) were added at a concentration of 10 μg/ml at the beginning of MLR. Where indicated, MLRs used BM-DC from BALB/c mice and CD4$^+$ splenocytes from *Il27ra*$^{-/-}$, *Il10*$^{-/-}$ or wild-type (C57BL/6) mice.

Adoptive transfer experiments. Wild-type BM-DC, BM-DC_{Gal1} or *Lgals1*$^{-/-}$ BM-DC were pulsed overnight with OVA (200 μg/ml; Sigma) and then were adoptively transferred (3×10^5 cells per mouse) into wild-type, *Lgals1*$^{-/-}$, *Il27ra*$^{-/-}$ or *Il10*$^{-/-}$ recipient mice by single or coinjection protocols. After 7 d, mice were injected subcutaneously with OVA (100 μg) in CFA; 7 d later, splenocytes were analyzed for proliferation and cytokine production after *ex vivo* restimulation with OVA (75 μg/ml).

Tumor-protection assays. B16 melanoma cells were subjected to four cycles of freezing-thawing for the preparation of lysates as described[23]. Mice (C57BL/6) were immunized twice subcutaneously at 7-day intervals with tumor lysate–pulsed or unpulsed DCs or DC_{Gal1} (1×10^6) by single or coinjection protocols. At 14 d after the final immunization, mice were challenged subcutaneously with 2×10^5 viable B16 cells. Tumor development was monitored as described[17]. Mice with tumor volume of less than 0.5 cm^3 were considered tumor free for the Kaplan-Meier analysis. For ethical reasons, mice were killed when tumors reached

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a volume greater than 2.5 cm^3. At 2 weeks after tumor challenge, lymph node cells (5×10^5 cells/well) were restimulated for 72 h with 1×10^4 irradiated (4,000 rads) B16 cells and were analyzed for proliferation and cytokine production.

Induction and assessment of EAE. EAE was induced in female C57BL/6 mice by subcutaneous immunization with 200 μg MOG(35–55) (MEVGWYRSPFSRVVHLYRNGK; Peptides International) in CFA supplemented with *Mycobacterium tuberculosis* (4 mg/ml; strain H37Ra; Difco). On days 0 and 2, mice received 200 ng pertussis toxin (List Biological Labs). Mice were assigned scores as follows: 1, limp tail; 2, hindlimb weakness; 3, hindlimb paralysis; 4, hindlimb and forelimb paralysis; and 5, moribund. Mice with established EAE (clinical score, 1) were injected intraperitoneally with 2×10^5 syngeneic MOG(35–55)-pulsed or unpulsed DCs or DC_{Gal1}. On days 25–30, spinal cords sections were stained with hematoxylin and eosin and with Luxol fast blue as described[19]. Proliferation and cytokine production were analyzed in lymph node cells as described[19] after *ex vivo* restimulation with MOG(35–55). For analysis of their migratory capacity, DCs were labeled with carboxyfluorescein diacetate succinimidyl ester (Molecular Probes) before injection. $CD11c^+$ cells were isolated at day 21 from spleens of immunized mice (purity, >96%) with MicroBeads (MACS; Miltenyi Biotec).

Statistical analysis. Prism software (GraphPad) was used for statistical analysis. Two groups were compared with the Student's *t*-test for unpaired data. Kaplan-Meier analysis was used for analysis of tumor protection assays. The Mann-Whitney U-test was used for clinical scores. *P* values of 0.05 or less were considered significant.

54. Sarter, K. *et al.* Detection and chromatographic removal of lipopolysaccharide in preparations of multifunctional galectins. *Biochem. Biophys. Res. Commun.* **379**, 155–159 (2009).